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COMPARATIVE STUDIES OF MINERAL CONSTITUENTS OF MARINE SPONGES

I. THE GENERA *Dysidea, Chondrilla, Terpios*[1]

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ABSTRACT

Analyses of Al, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sn, Ti, and V are presented for specimens of five species of marine sponges: *Dysidea crawshayi, D. etheria, Chondrilla nucula, Terpios fugax* and *T. zeteki;* and of F in *D. crawshayi.*

From replicate analyses on unhomogenized material from each specimen, and from comparison with such analyses made on other sponges from the same habitat and on the same species of sponge from other habitats, it is concluded that decision can be made as to whether a given element is truly a constituent of the sponge or of the living or dead extraneous matter which is unavoidably a part of each specimen.

From such comparisons it is concluded that *D. crawshayi* is an accumulator of F, K, and Ti, that *D. etheria* is not an accumulator of any of the elements studied, that *Chondrilla nucula* is an accumulator of Cu and Na, that *Terpios fugax* and *T. zeteki* are accumulators of Ti, and of K to some extent, and that *T. zeteki* may also accumulate Mn, Sn and V.

It is shown that one collection of *D. crawshayi* is characterized by an extraordinary level of Ni accumulation (F = 5×10^5 on a wet basis), which may be due to the

[1] Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

activities of an irregularly occurring microfloral inhabitant. From analyses of preservative solutions of museum specimens, it is concluded that this sort of Ni accumulation is not of infrequent occurrence in these sponge species and that the mass of accumulated material is in extractable form.

INTRODUCTION

As pointed out by Bergmann (1949), it has long been assumed that evolutionary divergence within the phylum Porifera has taken a clearly chemical form. The major divisions of the phylum have been based on the organic and inorganic composition of the skeletal material. Recently Low (1949) has shown evidence for a major division based on divergence in halogen content, and Bergmann (1949) has laid the groundwork for reclassification of some genera on the basis of sterol content. During the latter investigation, Bergmann called the senior author's attention to peculiarities of the ash of many of his sponges. The opportunity to study the mineral composition of a large series of carefully determined animals was a welcome one, especially since many other aspects of their biochemistry were being examined, and since, as an initial assumption, their geochemical environment was taken to be quite uniform.

Reported below are analyses on specimens of the genera *Dysidea*, *Terpios* and *Chondrilla*. This is the first of a planned series of studies of which the next will be a spectrographic survey of each of the specimens studied by Bergmann; work has already begun on this. This work will be followed in turn by detailed quantitative studies of all forms showing unusual features of mineral composition.

The choice of the genera *Dysidea*, *Terpios* and *Chondrilla* has a purely historical basis. The ash of *Dysidea crawshayi* from Walsingham Pond, Bermuda, was the first sample shown to us by Bergmann, it having caught his attention by its extraordinary fusibility and bright green color; at that time the only other specimen available from Walsingham Pond was *Terpios fugax*. *Chondrilla nucula* from Harrington Sound, Bermuda, was chosen as a sponge which had no known biochemical peculiarities and which had not been exposed to the peculiar environment of Walsingham Pond. Other specimens and species were added subsequently to provide additional comparisons.

The organic chemists who have studied sponge composition (Bergmann, 1949; Low, 1949) have used the nonspicule ash as an indication of the amount of extraneous material in the sample. But it appears to us that a more detailed consideration of the exogenous components of a sponge sample is justified. It is commonplace in biological thought that the mineral components of organisms play an essential

part in their functioning and that they show, between species or larger groups, variations which are quite as consistent and often quite as inexplicable as those shown by the organic components. It seems evident from such studies as those of Pearse (1950) and de Laubenfels (1950a), coupled with the work of Bergmann and Low, that the mineral elements in a given sponge sample must be thought of as having five sources: the sponge tissue itself, macrodetritus, microdetritus, macro-organisms inhabiting the sponge, and micro-organisms inhabiting the sponge. Gross examination shows that there is considerable irregularity in distribution within each specimen, both of the macrodetritus and of the macroinhabitants. Accordingly, it may be thought that chemical constituents which show the same sort of irregularity between replicate analyses of unhomogenized tissue are to be referred either to macrodetritus or to macroinhabitants.

On the other hand, we should not expect exogenous material that enters as microdetritus (of which coral sand in these sponges is thought to be a major component) to show wide fluctuations between duplicate analyses, nor should we expect the content of micro-organisms to vary widely among specimens of one species caught at the same place and time. However, it does seem reasonable to assume that the composition, and, to a degree, even the amount of microdetritus, will be common to sponges of different species taken at the same place and time, whereas we should not expect the faunae and florae of different genera, or even species, to be highly alike. Thus we are inclined to conclude that elements which show uniform distribution among replicates of the same sample but which are not found in similar concentration in other species from the same habitat are true constituents of the sponge or have been introduced in the bodies of the micro-organism population.

It appears from de Laubenfels' (1905a) detailed description of Bermuda sponges that this discussion may require modification due to the fact that some sponges select their detrital components. Thus, *Dysidea etheria* reportedly accumulates calcareous detritus almost exclusively, whereas *D. crawshayi* accumulates largely silicious spicules of other sponges. Pursuit of extensive field studies, combined with analyses of material which is made available for microscopic examination in fairly fresh condition, will probably have to determine how far this property of selection is general among sponges.

However, there does not appear to be adequate information as to the extent to which the micro-organism population of a sponge species may be subdivided into two portions: one facultative, characterizing only a given habitat, the other obligate, characterizing the species no matter where collected. Although some sponge species have been

regarded as distinct (Verrill, 1907; Topsent, 1929) on the basis of their inhabitants (algae in these two cases), de Laubenfels' (1950a, 1950b) comparisons in the field did not lead him to decide that any of the Bermuda sponges is characterized by a particular micro-organism population. Should such a population be found, we do not discern any mechanism whereby comparison analyses can be used to differentiate its chemical contribution in a sponge sample from that of the sponge tissues *sensu strictu*. Both contributions will be uniformly distributed among replicates, will not be common to other sponge species from the same habitat, and will be common to all collections of the species in question. On the other hand, contributions by the "facultative micro-organism inhabitants" will evidently be characterized by uniform distribution among replicates of the same sample and by dissimilar concentrations in specimens of other species from the same habitat and of the same species from different habitats. This reasoning, of great importance to the conclusions set out below, is summarized in Table IV.

Thus, for maximum elucidation of analytical data on specimens of unavoidably high contamination, it appears that analyses must be made on as many species as possible from each collecting point, on specimens of each species from as many collecting points as possible, and on several replicate samples taken from each specimen without previous attempts to render the sample homogeneous. That this proves to be a difficult regime to follow in the case of tropical sponges is indicated by the few sets of comparison analyses which actually could be made, but we feel that the results set out below justify both the reasoning and the attempt to follow its consequences.

Although some of these conclusions are implicit in the thoughtful discussion by Webb (1937) on the mineral constituents of marine organisms, and although Fox and Ramage (1931) used comparison analyses to confirm the validity of nickel accumulation as a characteristic of the annelid genus *Myxicola* (see p. 163), neither study was planned to take advantage of the approach we have outlined.

METHODOLOGY

The specimens analyzed were mostly in the form of residues after acetone extraction, as described by Bergmann (1949). The analytical figures for these have been restored to a whole dry weight basis, using Bergmann's figures for organic fat. In addition, some specimens, originally donated by Bergmann, were obtained from the Bingham Oceanographic Collection. These had been preserved in alcohol or formalin and were dried before analysis. Some analyses were made on the fluid extracts as well.

Both dry and wet ashing have been employed. In the former process the dry material, in a clean weighed porcelain crucible, was placed in an electric muffle furnace where the temperature was slowly raised to 200° C, held at this point from 6 to 12 hours, then slowly raised to 500° C and held at this point from 4 to 6 hours. Clean ash of low carbon content was obtained.

Wet ashing, in pyrex vessels of suitable size, was effected generally with nitric and perchloric acids, mostly in the following proportions: 5 ml HNO_3 and 3 ml $HClO_4$ to 1 g dry sponge. Some large samples of *Dysidea crawshayi* had potassium too high for convenient perchloric treatment, and these were treated with nitric and sulfuric acids in the following proportions: 5 ml HNO_3 : 1 ml H_2SO_4 : 1 g dry sponge. Sample sizes varied from 0.5–20.0 grams, and in general ashing proceeded smoothly.

The analytical methods employed have been quite varied. Through the cooperation of Morris Slavin of Brookhaven National Laboratory, survey spectrograms were obtained by arcing dry ash in carbon electrodes with a Baird Associates 1 meter grating spectrograph. The semiquantitative interpretation of these spectrograms is due to Mr. Slavin's long experience, and we take pleasure in extending our thanks to him.

Standard procedures, substantially as described by Hillebrand and Lundell (1929), were used in determining silicon by perchloric acid dehydration; aluminum with ammonia following cupferron separation of other R_2O_3 cations; and calcium as oxalate.

Magnesium was determined, following the other separations, either as the ammonium phosphate or as the 8-hydroxy quinolate.

Sodium and potassium were determined on hydrochloric acid extracts of dry ash with the Beckman flame spectrophotometer.

Iron was determined photometrically as ferrous dipyridyl, substantially as outlined in Sandell (1944). Although the orthophenanthroline method is somewhat more sensitive, the great insolubility of ferrous orthophenanthroline perchlorate proved an overpowering disadvantage.

Copper was determined with diethyldithiocarbamate substantially as outlined in Sandell (1944).

Cobalt was determined with nitrosocresol according to the extremely sensitive method of Ellis and Thompson (1945).

Nickel was determined both gravimetrically with dimethylglyoxime, and colorimetrically with diethyldithiocarbamate, following preliminary extraction with dimethylglyoxime (Alexander, Godar and Linde, 1946). The latter method proved satisfactory and was capable of extreme sensitivity.

TABLE I

Analyses in Terms of Whole Dried Sponge

Species	Sample Place and Date	Ash %	SiO_2 %	Ca %	Mg %	Na %	K %	Al %	Fe %	Co ppm	Ni ppm	Mn ppm	Ti ppm	Cu ppm	Sn ppm	Mo ppm	Pb ppm	V ppm
Dysidea crawshayi	A-1-1 Dried Specimen Bermuda 1946	34.1	1.08^{g}	4.79^{g}	0.93^{g}	3.29^{f}	4.11^{f}	1.73^{g}	0.067^{c}	--	23^{c} 22^{c} 24^{c}	--	--	22^{c} 21^{c}	--	--	--	--
Dysidea crawshayi	A-1-2 Extracted Specimen Walsingham Pond Bermuda 1946	32.5	1.6^{s} 0.11^{g} 0.31^{g}	1.18^{g} 0.53^{g}	0.77^{g} 0.49^{g}	3.25^{f} 3.25^{f}	7.10^{f} 7.70^{f}	0.29^{g}	0.25^{c} 0.27^{g}	1.6^{c} 2.2^{c} 2.7^{c}	614^{g} av.	31^{v}	127^{c} 137^{c} 150^{c}	298^{c} 281^{c}	nil^{s}	--	nil^{s}	nil^{s}
Dysidea crawshayi	A-1-3 Extracted Specimen Walsingham Pond Bermuda 1947	32.8	1.6^{s}	0.78^{g}	--	2.79^{f} 2.85^{f}	7.56^{f} 7.15^{f}	0.19^{g}	0.35^{c} 0.11^{c}	nil^{c} 0.1^{c}	1.8^{c} 2.7^{c}	11^{c}	90^{c} 100^{c} 116^{c}	38^{c} 39^{c} 43^{c}	nil^{s}	--	3^{s}	3^{s}
Dysidea etheria	A-1-4 Extracted Specimen Bimini, Bahamas 1948	75.6	0.23^{g}	30.20^{g}	0.32^{g}	9.46^{f}	0.38^{f}	0.59^{g}	0.03^{c}	nil^{c} nil^{c}	1.8^{c} 2.4^{c}	25^{c}	<10^{c}	10^{c} 15^{c}	7^{s}	0.7^{s}	nil^{s}	<1^{s}
Dysidea etheria	A-1-5 Dried Specimen Bermuda 1946	30.8	2.45^{g}	8.04^{g}	0.60^{g}	3.55^{f}	0.39^{f}	1.85^{g}	0.28^{c} 0.34^{c}	0.5^{c}	7.3^{c} 8.0^{c}	59^{v}	215^{c} 270^{c} 300^{c}	60^{c}	nil^{s}	--	3^{s}	nil^{s}
Chondrilla nucula	A-8-1 Extracted Specimen Harrington Sound Bermuda 1946	30.5	1.72^{g} 1.73^{g} 1.82^{g}	11.35^{g} 8.95^{g}	0.66^{g} 0.75^{g} 0.75^{g}	6.68^{f}	0.77^{f}	0.06^{g} 0.27^{g} 1.24^{g}	0.98^{c} 1.11^{c}	~.1^{c} ~.1^{c}	18^{c} 18^{g} 20^{c}	45^{c} 42^{v}	<10^{c}	385^{c} 397^{c}	--	--	--	--
Chondrilla nucula	A-8-2 Extracted Specimen Bermuda	40.4	1.18^{g}	3.43^{g}	0.73^{g}	7.50^{f}	0.60^{f} 0.61^{f}	0.59^{g}	0.045^{c} 0.057^{c}	1.5^{c} 1.5^{c} 2.1^{c}	67.5^{c} 68.0^{c}	10^{c} 40^{c}	<10^{c}	324^{c} 330^{c} 339^{c}	4^{s}	--	nil^{s}	nil^{s}
Terpios fugax	A-5-2 Extracted Specimen Walsingham Pond Bermuda 1946	30.1	15.45^{g}	1.15^{g} 2.05^{g}	0.40^{g}	2.10^{f} 2.84^{f}	1.0^{f} 1.2^{f}	0.11^{g} 0.12^{g} 0.70^{g}	0.076^{c} 0.10^{c}	~.1^{c}	18^{c} 20^{c}	15^{c}	88^{c} 97^{c} 100^{c}	56^{c} 61^{c} 62^{c} 66^{c}	--	--	--	--
Terpios zeteki	A-5-3 Extracted Specimen Hawaii 1948	54.5	39.80^{g}	1.52^{g} 2.32^{g}	0.42^{g}	2.55^{f} 2.62^{f}	0.80^{f} 0.93^{f}	0.13^{g} 0.41^{g}	0.14^{c} 0.15^{c} 0.20^{c}	0.1^{c} 0.8^{c}	2.9^{c} 4.9^{c}	253^{c} 200^{c}	96^{c} 120^{c} 180^{c}	50^{c} 60^{c} 72^{c} 84^{c}	50^{s}	--	nil^{s}	5^{s}

Superscripts: s = spectrographic c = colorimetric g = gravimetric v = volumetric f = flame spectrophotometric --: not determined

Manganese was determined as permanganate either by arsenite titration after persulphate oxidation (Hillebrand and Lundell, 1929) or spectrophotometrically after periodate oxidation (Sandell, 1944).

In all of the above-mentioned colorimetric methods, a Beckman model D. U. spectrophotometer was employed.

Titanium was determined with peroxide (Sandell, 1944). A preliminary separation with cupferron served to remove interference except for that due to iron. The positive error due to iron in a hydrochloric acid solution was corrected by running blank samples to which iron had been added to equal that of the specimens. For this method a Lumetron electrophotometer with a 440 mμ medium band filter and 50 mm cells was used.

RESULTS

The main body of data is presented in Table I. In accordance with the arguments outlined above, the replicate figures entered in many places are results of analyses on replicate samples taken without previous attempt to render the sample homogeneous. In many cases the figure entered is actually the mean of two or more analyses made on aliquot portions of such replicate samples, thus reducing to a minimum the possible significance of purely analytical variation.

The analyses for silica on *Dysidea crawshayi* are complicated by the extraordinary fluorine content of this species. Detailed consideration of sponge fluorine must await a later report, but all specimens of this species encountered thus far show fluorine in the neighborhood of one per cent of the dry sponge. It is thought that this may interfere less with the spectrographic silicon estimations which are included and which do indeed show a much higher figure than has been obtainable chemically.

The calcium figures include strontium. According to present plans, we intend to present data for the Ca : Sr ratios of these and of other sponges at a later date.

As noted previously, the green ash of *D. crawshayi*, taken from Walsingham Pond in 1946, was the starting point of this investigation. The nickel concentration in this specimen was so extraordinary that when the same species taken from the same place in the following year was found to be low in nickel, some consternation was experienced. As discussed hereafter, replicate analyses on the high nickel specimen did not support the possibility of contamination in handling; however, it was thought that this possibility might be discounted further by examination of the preserved specimens which had been deposited in the Bingham Oceanographic Collection, since their treatment had not included drying or acetone extraction, the two

TABLE II. Analyses of Museum Specimens

Species	Place and Date	Preservative	Preservative: Ash as % Dry	Preservative: Ni as ppm Dry	Specimen: Ash as % Dry	Specimen: Ni ppm Dry	Specimen: Cu ppm Dry
Dysidea etheria	Virginia Key, Florida, 1945	Formaldehyde	64.2	<1	—	—	—
Dysidea etheria	Mullets Bay, Bermuda, 1946	Alcohol	48.2	nil	63.9	2.0	—
Dysidea fragilis	Plymouth, England	Alcohol	47.3	nil	75.5	4.6	—
Dysidea crawshayi	Walsingham Pond, Bermuda, 1946	Alcohol	23.6	nil	34.6	2.5	—
Dysidea crawshayi	Harrington Sound, Bermuda, 1946	Alcohol	23.5	nil	28.5	3.4	—
Dysidea crawshayi	Virginia Key, Florida, 1945	Formaldehyde	64.6	<0.5	—	—	—
Terpios fugax	Walsingham Pond, Bermuda, 1946	Alcohol	38.1	4	27.7	13.6	20.3
Chondrilla nucula	Harrington Sound, Bermuda, 1946	Alcohol	32.8	~1	48.6	8.9	22.2

principal manipulations to which the main sample had been subjected. In Table II are presented the analyses made on the group of museum specimens chosen at this time. Although interesting, these certainly gave no indication of the high nickel that was so characteristic of the 1946 *D. crawshayi*.

As a consequence of curatorial aesthetics, however, the preservative on marine specimens is changed regularly until no more alcohol-precipitable material is extracted. We could obtain no estimate of the number of changes and extractions to which this material had been subjected, but it was known that the *Terpios* and *Chondrilla* preservatives were at least one change behind the others, and these showed the highest nickel content. To check further on the interesting hypothesis that nickel in sponges may be largely in extractable form, another series of solutions was obtained from the museum specimens. This series of fluid preservatives or extracts represented material which had been replaced by fresh preservative on the samples of Table II. The analyses of these preservatives are presented in Table III. As ex-

TABLE III. Analyses of Preservative from Museum Specimens

Species	*Place and Year*	*Solution*	*Ash% Dry Wt.*	*Ni ppm Dry*	*Cu ppm Dry*
Dysidea crawshayi	Walsingham Pond, Bermuda, 1946	Alcohol	24.3	8.0	323
Dysidea crawshayi	Harrington Sound, Bermuda, 1946	Alcohol	21.3	18.3	470
Dysidea crawshayi	Virginia Key, Florida, 1945	Formalin	64.6	2.3	51
Dysidea etheria	Harrington Sound, Bermuda, 1946	Alcohol	52.5	8.0	1288
Dysidea etheria	Mullets Bay, Bermuda, 1946	Alcohol	50.7	2.2	494
Dysidea etheria	Virginia Key, Florida, 1945	Formalin	57.3	19.4	289
Chondrilla nucula	Harrington Sound, Bermuda, 1946	Alcohol	39.9	7.0	1010
Terpios fugax	Walsingham Pond, Bermuda, 1946	Alcohol	38.0	7.0	435
Terpios fugax	Walsingham Pond, Bermuda, 1947	Alcohol	32.5	3.5	750
Terpios fugax	Harrington Sound, Bermuda, 1946	Alcohol	30.7	15.0	1330

pected from the earlier position of these samples in the extraction series, their content in nickel is considerably higher. Further discussion of the significance of these preservative analyses will be found in the following discussion.

DISCUSSION

If we now apply the conclusions reached in the INTRODUCTION (summarized in Table IV) to an analysis of the data in Table I, we find certain patterns in each of the five species selected.

Dysidea crawshayi, presently assigned to a genus characterized by lack of silica accumulation, shows rather consistently that there is more silica than seems reasonable. We have already discussed the reason for our rejection of the low silica figures generally obtained gravimetrically for this species. In addition, a high potassium content is shown uniformly, even by specimen A-1-1 which was air dried

TABLE IV. USE OF COMPARISON ANALYSES TO ELUCIDATE ORIGIN OF CHEMICALS ANALYZED IN SAMPLES HAVING HIGH AMOUNTS OF EXOGENOUS MATERIAL

Origin of Chemical Component analyzed	*Constancy among replicates of one specimen*	*Constancy among specimens from one habitat*	*Constancy among samples of one species from different habitats*
Sponge tissue, *sens. str.*	high	low	high
"Obligate micro-organism population"	high	low	high
"Facultative micro-organism population"	high	low	low
Macro-organism population	low	low	low
Microdetritus	high	high	low
Macrodetritus	low	low	low

and probably had lost much of this element. The two specimens analyzed show agreement in that they gave consistent and rather high titanium. Most other elements show variations indicating origin from detritus or from "macroinhabitants" or "facultative microinhabitants." For these specimens the case of nickel is discussed fully.

Dysidea etheria, whose variable silica figures indicate that this element is of exogenous origin, differs also from its congener above in being low in potassium. The great variability of the other constituents apparently indicates that this species possesses an unusually high content of exogenous matter.

Chondrilla nucula, which gives the consistent silica figures that are to be expected of a silicious skeleton, has uniformly high sodium, moderate potassium and magnesium, uniformly high copper, and very low titanium. The iron of our two samples shows an amazing variation.

The genus *Terpios*, of which we have only a single specimen for each of the two species, shows the high SiO_2 of a very silicious sponge. Also, t shows moderately high and quite consistent potassium and titanium,

and rather low but consistent copper. *T. zeteki* shows a high manganese content, whereas *T. fugax* is among the low manganese samples.

For these five species then, we may add the following observations in addition to the well-known fact that sponges accumulate calcium and silicon. One species, *D. crawshayi*, may be characterized by an accumulation of titanium and potassium, and of fluorine as mentioned previously. Another, *C. nucula*, may be characterized by accumulation of much copper, and both species of *Terpios* may be characterized by accumulation of titanium and of moderate but significant amounts of copper. We conclude that all of these elements are accumulated by the activities of the sponge itself or of any "obligate microinhabitants."

In addition, accumulations which appear to be due to the activities of "facultative microinhabitants" are found for aluminum, iron, copper, nickel and titanium. High levels of manganese, tin and vanadium were found, but insufficient data prevent assignments of origin.

Nickel. The best previous nickel determination in a marine sponge (Noddack and Noddack, 1939) gave 22 ppm of the dry specimen for *Halichondria* from the North Sea. Fox and Ramage (1931), in their examination of two specimens of *Clathrina coriacea*, evidently found no nickel. In polychaete worms and mollusks, Fox and Ramage (1931) found nickel quite generally, with evidence that *Myxicola* of the former group is characterized by nickel accumulation that runs from 80 to 100 ppm dry regardless of the locality; however, in *Haliotis* foot only, high nickel (40 ppm) was found in the absence of high cobalt. Our finding of 614 ppm nickel (the mean of several highly concordant replicate analyses) in dried *Dysidea crawshayi* from the 1946 collection at Walsingham Pond evidently indicates something quite new in nickel metabolism. The possibility that this nickel may be due to contamination introduced in handling seems contraindicated both by what is known of the manipulations involved and by the extreme uniformity with which nickel is distributed through a specimen of about two kilograms. At present we are inclined to conclude that the *D. crawshayi* population of Walsingham Pond, in 1946, was infested by a microflora characterized by an extraordinary level of nickel accumulation. The reasoning that leads to this conclusion is as outlined previously, plus the added assumption that plant accumulation of nickel is more likely in view of the general tendency toward nickel selection by the plant kingdom (Hutchinson, 1949). It is seen in our analyses (Table I) that there is no strong tendency for cobalt to vary in relation to nickel.

As mentioned previously, precise interpretation of the analyses set out in Tables II and III is difficult because of the uncertainty as to the

number of extractions that preceded the extractives. It does appear highly probable, however, that a large fraction of the nickel present in *D. crawshayi* from Walsingham Pond, 1946, was alcohol-extractable. If we accept this conclusion, such analyses as those on *D. crawshayi* from Harrington Sound, 1946, on *T. fugax* from Harrington Sound, 1946, and on *D. etheria* from Virginia Key, 1945, may indicate that similar infestations of nickel-accumulating plants characterized these populations. Similar conclusions for copper are indicated from Table III. Collections now being made by the personnel of the Bermuda Biological Station under conditions conducive to the most precise control of mineral content are expected to provide confirmation of this variable in nickel uptake by marine sponges. In time we hope that concomitant water collections will even reveal whether the nickel organisms are inhabitants of the sponge or whether they are planktonic and appear in selected sponges as a result of the sort of differential predation postulated by Bertrand (1950) in the case of vanadium accumulation by tunicates.

Accepting the figure of 0.5×10^{-9} for the nickel content of sea water, as given by Noddack and Noddack (1939), the concentration factor of approximately 5×10^5 on a wet basis for the high nickel *D. crawshayi* appears to indicate extreme intervention of this element in the metabolism of some organisms. For comparison we may cite the accumulation of vanadium by tunicates, which involves concentration factors in the range of 1 to 3×10^5 (Bertrand, 1950).

Here it may be worth pointing out that no rational program for marine disposal of radioactive waste is possible in the absence of data much more detailed than that presented here. In the case of strontium, the studies of Spooner (1949), coupled with those of Odum (1950), indicate that high concentration factors are rare among marine organisms; furthermore, they indicate that the high content of strontium in sea water may be expected (1) to provide an immediate reduction to a safe level in specific activity of released strontium isotopes and (2) to prevent the operation of any local geochemical isotope concentration mechanisms. In the case of almost no other long-lived radioisotopes which present a disposal problem do we have sufficient analyses of sea water or of biological concentration phenomena to permit an adequate judgment of safe levels.

Further, it appears evident that too much thinking on waste disposal problems has viewed the ocean as a continuum and has assumed that the entire mass of a given element in the ocean is to be viewed as immediately effective as a diluent. Scott (1950) indicates local bottom accumulations as the prime exception. That this is not a reasonable assumption is indicated by such studies as those of Cooper

(1935) and Harvey (1947) which show that locally high fractions of the available iron or manganese may be present at times in the bodies of plankton. The local massive accumulations of nickel and of some other elements which are suggested in the present study pose an even more disturbing problem, since we are uncertain how general they may be, both with respect to the elements and to the species concerned. Data must be accumulated in this direction as soon as possible, as well as in the direction of ascertaining how such accumulations affect uptake of elements by organisms associated environmentally with the accumulator. In the case of nickel, this is made especially interesting by the solubility of the accumulated element, bringing to mind the toxicity-enhancing properties of those selenium accumulators which release soluble selenium compounds to the soil (Trelease and Beath, 1949).

ACKNOWLEDGMENT

It is a great pleasure to acknowledge the interest and cooperation of Professor Werner Bergmann, who has supplied generous samples from his collection and through whose good offices the taxonomic assistance of Professor Max W. de Laubenfels of Oregon State University was obtained. Dr. Willard Hartmann has been generous in procuring museum specimens from the Bingham Oceanographic Collection and from Peabody Museum of Yale University and has provided much stimulating discussion of sponge biology and museum practice.

Throughout this study Professor G. E. Hutchinson has been a source of support and stimulation. The investigation was begun at Yale University during the senior author's appointment as Research Assistant in Biogeochemistry under the auspices of the American Museum's Survey of Contemporary Knowledge of Biogeochemistry, whose support is gratefully acknowledged. The present study has been completed at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

SUMMARY

Analyses of Al, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sn, Ti, and V are presented for specimens of five species of marine sponges: *Dysidea crawshayi*, *D. etheria*, *Chondrilla nucula*, *Terpios fugax* and *T. zeteki;* of F in *D. crawshayi.*

From replicate analyses on unhomogenized material from each specimen, and from comparison with such analyses made on other sponges from the same habitat and on the same species of sponge from other habitats, it is concluded that decision can be made as to whether a given element is truly a constituent of the sponge or of the living or

dead extraneous matter which is unavoidably a part of each specimen.

From such comparisons it is concluded that *D. crawshayi* is an accumulator of F, K, and Ti, that *D. etheria* is not an accumulator of any of the elements studied, that *Chondrilla nucula* is an accumulator of Cu and Na, that *Terpios fugax* and *T. zeteki* are accumulators of Ti, and of K to some extent, and that *T. zeteki* may also accumulate Mn, Sn and V.

It is shown that one collection of *D. crawshayi* is characterized by an extraordinary level of Ni accumulation ($F = 5 \times 10^5$ on a wet basis), which may be due to the activities of an irregularly occurring microfloral inhabitant. From analyses of preservative solutions of museum specimens, it is concluded that this sort of Ni accumulation is not of infrequent occurrence in these sponge species and that the mass of accumulated material is in extractable form.

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NOTES ON THE DEVELOPMENT OF A THERMISTOR TEMPERATURE PROFILE RECORDER (TPR)

By

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ABSTRACT

A vertical temperature profile recorder, utilizing a thermistor as the temperature sensing element, has been developed for use on lakes, bays, or other enclosed or semi-enclosed bodies of water where temperature profiles in the upper 30 m are desired. Tests of the instrument indicate that it is rugged and durable, giving easily readable records accurate to ± 0.1° C. The response factor of the composite instrument (thermistor, amplifier, and recorder) is 1.4 seconds. To obtain maximum accuracy, the instrument must be lowered at a rate compatable with the magnitude of the temperature gradient; that is, the larger the gradient the slower must be the rate of lowering. Since depth is recorded by measuring the amount of paid-out cable, its use is restricted to conditions of zero wire angle.

In connection with the prosecution of the Lake Mead Water Loss Investigations, being conducted jointly by the U. S. Navy Electronics Laboratory (NEL), U. S. Geological Survey, U. S. Bureau of Reclamation, and U. S. Weather Bureau, it was necessary to develop a portable instrument for obtaining vertical temperature profiles to a maximum depth of 30 m. The instrument was designed for use on lakes, bays, or other enclosed or semi-enclosed bodies of water where wind-wave, swell, and surface currents are at a minimum. This instrument may have application to various problems in physical, chemical, and biological limnology and in estuarian oceanography where suitable conditions exist for its operation.

DESCRIPTION OF THE INSTRUMENT

The instrument, illustrated by Fig. 1, consists primarily of a hand-operated cable reel, Esterline-Angus recorder, and amplifier. It utilizes a Western Electric thermistor (type 14B) as the temperature-sensitive element. Since the instrument was designed for use in relatively protected bodies of water, the depth is obtained by measuring the amount of paid-out cable. This is accomplished by connecting the cable reel mechanically to the paper drive on the Esterline-Angus recorder in such a manner that one turn on the cable reel, which pays out one meter of cable, moves the recorder paper one division. The instrument is designed to cover five temperature ranges: 0–50° C,

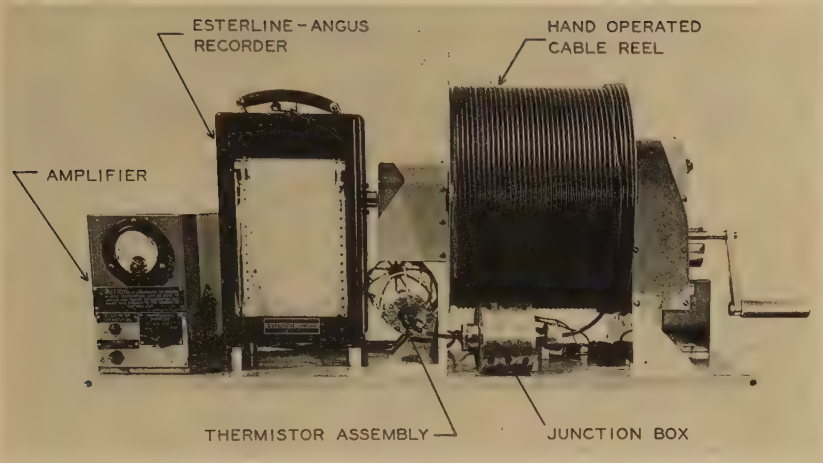

Figure 1. Temperature Profile Recorder showing the various components.

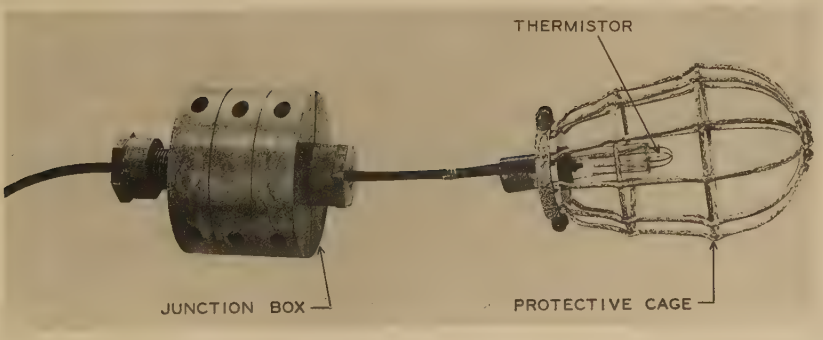

Figure 2. Thermistor assembly.

0–10° C, 9–19° C, 18–28° C, and 27–37° C, with a calibrated accuracy of ± 0.5° C on the 50° C range scale and ± 0.1° C on the 10° C range scale. In order to permit slow lowerings of the thermistor through large temperature gradients, the hand-operated crank has a gear ratio of 9 : 1.

The thermistor assembly is illustrated by Fig. 2. The thermistor, encased in glass, is mounted in a plastic block which electrically insulates the thermistor lead connections from the water. A junction box for easy replacement of damaged thermistor units is provided. The instrument is designed to utilize three interchangeable thermistor units.

The thermistor is part of a direct current Wheatstone bridge which is so designed that the different temperature ranges are selected by a panel switch, which connects special precision resistors into the bridge so that at the lowest temperature of each range the bridge is exactly balanced. Because the thermistor changes resistance, at higher temperatures (in the given range) the bridge becomes unbalanced. If the voltage source to the bridge is kept at 1.2 volts, the unbalance voltage is a measure of the temperature of the thermistor. The unbalance voltage is amplified and measured on the Esterline-Angus recorder. The bridge resistors are made of manganin wire, which allows the recorder to work in average air temperatures without affecting the accuracy of the resistors.

The instrument has been designed to give maximum performance with the least current drain and expense practicable. Low current drain battery tubes and standard components are used almost exclusively. The entire instrument uses two batteries: a 1.5 volt type battery with a current drain of 20 milliamperes which energizes the Wheatstone bridge; and a 6.0 volt storage battery for energizing the amplifier with a current drain of about 1.2 amperes.

Fig. 3 is a schematic circuit diagram of the electronic portion of the instrument. The battery, B, supplies power for the Wheatstone bridge through voltage dividers, R_A and R_B. The voltage across R_B is adjusted to 1.2 volts by means of R_A and the small panel meter, M. The values of R_1, R_2, and R_3 for each range are manufactured so that when the temperature of the thermistor is at 0° C, 0° C, 9° C, 18° C, and 27° C (the low value of each of the range scales), the bridge is balanced and the voltage output, E_B, is zero. When the temperature of the water around the thermistor increases, the resistance of the thermistor decreases, unbalancing the Wheatstone bridge. E_B increases to some value which is a measure of the water temperature. For example, on the 0–10° C range scale, when the water temperature is exactly 10.0° C, E_B is 0.124 volt. This voltage is compared electronically with the

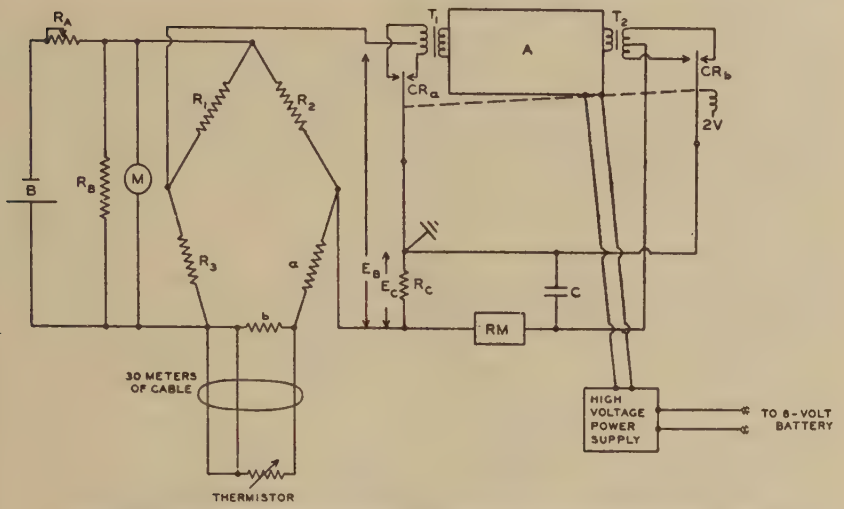

Figure 3. Schematic circuit diagram of Temperature Profile Recorder (TPR).

voltage across R_C, and the difference is changed to alternating current voltage by the synchronous vibrator arm, *CRa*, and amplified by the amplifier, *A*. The amplified voltage is rectified or demodulated by arm *CRb* and again becomes direct current voltage many times greater than the incoming voltage. This direct voltage causes a current to pass through the Esterline-Angus recorder, *RM*, and the resistor, R_C, generating a voltage E_C. The system is so designed that the voltage E_C tries to become the same as E_B at all times. (In reality it is a servo-system.) Thus, for the 0–10° C range, $R_C = 124$ ohms. When $E_B = 0.124$ volt, E_C becomes practically 0.124 volt and the current through the Esterline-Angus recorder is one milliampere or full scale. The recording meter thus indicates 0 milliampere for 0° C, one milliampere for 10° C, and proportionally between those temperatures on the 0–10° C range.

RESPONSE FACTOR

The instrument is designed to provide a continuous record of the vertical temperature profile. In order to be certain that the instrument is actually recording the *in situ* temperature, it was necessary to investigate the response factor (τ) of the instrument when the thermistor is exposed to various temperature gradients. In order to obtain reasonable lowering rates of the thermistor, this response factor must be relatively small.

Newton's law of cooling states that

$$\frac{d\vartheta}{dt} = \frac{\vartheta_a - \vartheta}{\tau}, \tag{1}$$

where ϑ is the temperature of the thermistor at time t, ϑ_a is the temperature of the water, and τ is the response factor.

Solving equation (1),

$$\frac{\vartheta - \vartheta_a}{\vartheta_0 - \vartheta_a} = e^{-t/\tau}, \tag{2}$$

where ϑ_0 is the temperature of the thermistor at $t = 0$.

Equation (2) may be rewritten as follows:

$$\frac{\vartheta_0 - \vartheta}{\vartheta_0 - \vartheta_a} = 1 - e^{-t/\tau} = R. \tag{3}$$

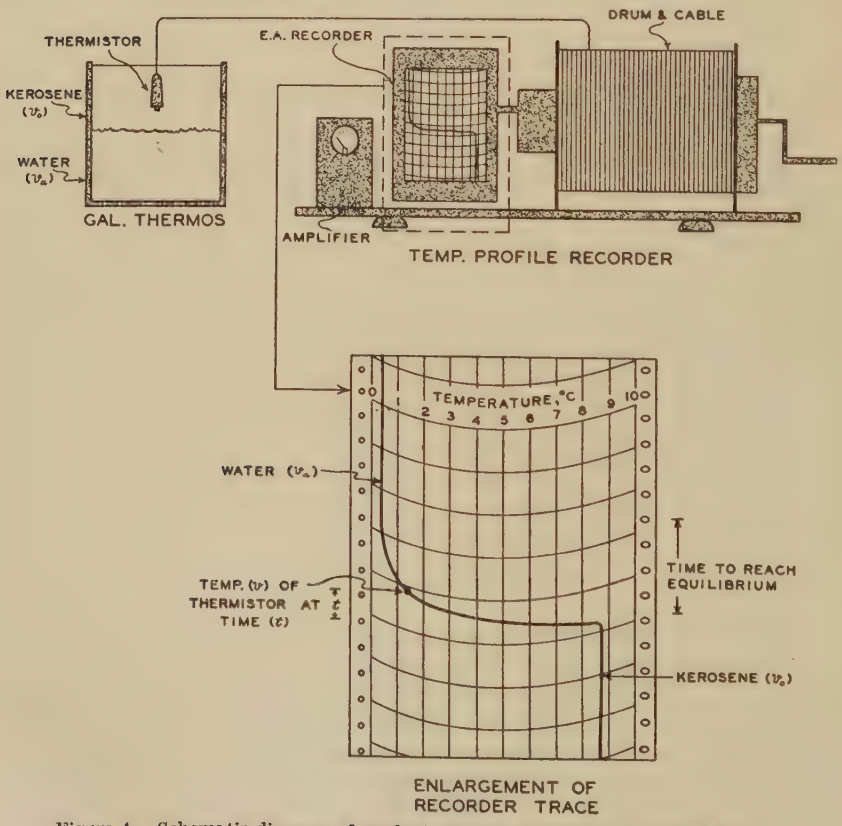

Figure 4. Schematic diagram of method used for determining response factors.

If the thermistor, at temperature ϑ_0, is suddenly moved into water at temperature ϑ_a, then its relative response at time t is given by equation (3). This is the equation generally used for determining the response factor with a single change of the surrounding environment. When $t = \tau$, $R = 0.632$; or in other words, the response factor is the time it takes for the instrument to respond to 63.2% of a given temperature difference.

The method for obtaining the response factor for the complete instrument (thermistor, amplifier, and recorder) is illustrated schematically by Fig. 4. The thermistor was placed in a one-gallon thermos half filled with water at temperature ϑ_a and half filled with kerosene at temperature ϑ_0. Since the Esterline-Angus recorder used in the instrument is driven by the hand crank, this recorder was replaced by a similar clock-driven Esterline-Angus recorder. To obtain accurate values for t, the speed of the Esterline-Angus recorder drive was accurately determined. To obtain values of ϑ_a, ϑ_0, ϑ, and t, the thermistor was placed in the kerosene and was then plunged into the water with a resulting trace similar to that shown in Fig. 4. The response factor was then computed for the given set of conditions.

Table I summarizes the response factors for the entire instrument by utilizing three different thermistors and temperature differences ($\vartheta_0 - \vartheta_a$) varying from 0.9° C to 19.0° C. The average response factors for the system with thermistors No. 2, 3, and 11 were 1.5, 1.3, and 1.4 seconds respectively.

In order to obtain the response factor for the thermistor alone, the same method as that described above was used, except that the instrument's recorder and amplifier were replaced by a high-speed recorder and amplifier whose response factors were essentially zero.

TABLE I. Summary of Response Factor

	Determination for TPR		
Thermistor No.	*No. Trials*	$\vartheta_0 - \vartheta_a$	*Average (sec.)*
2	3	1.4° C	1.5
2	3	4.4	1.5
2	3	9.2	1.4
3	3	1.1	1.3
3	3	3.3	1.3
3	2	8.8	1.3
11	3	0.9	1.6
11	6	4.9	1.4
11	6	8.1	1.3
11	3	15.3	1.4
11	3	19.0	1.3

Thirteen response factor determinations for the thermistor alone were made by using the method described above. These resulted in an average value of 0.8 second. Using this value for the thermistor and a value of 1.4 seconds for the complete instrument, the response factor of the amplifier and recorder system is 0.6 second.

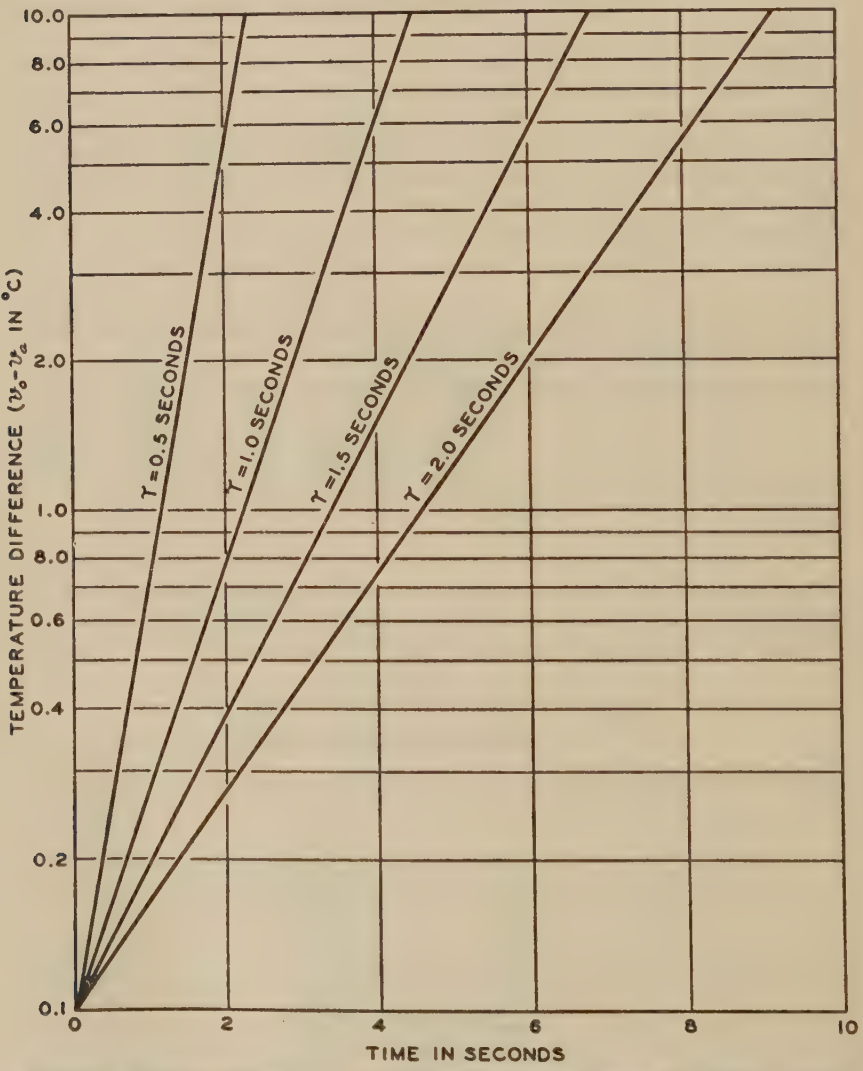

Figure 5. Time for reaching 0.1° C of a given temperature change for various response factors, τ.

As previously indicated, the response factor is the time it takes for the instrument to respond to 63.2% of a given temperature change. In using this instrument for making temperature profiles, it is necessary to know the time it takes to reach within 0.1° C of any given temperature difference. Fig. 5 presents curves of this time for various response factors and temperature differences. For $\tau = 1.5$ and $\vartheta_0 - \vartheta_a = 10°$ C, approximately 6.8 seconds are required for the instrument to respond to 9.9° C of the 10.0° C temperature change. In a practical sense this means that, if a thermocline of 10.0° C is present, the thermistor must be lowered through it in not less than 6.8 seconds in order to obtain temperature with an accuracy of ± 0.1° C.

In summary, the response factor of the instrument appears to be satisfactory when used under normal or average conditions; however, if steep thermoclines are present, it may be difficult to obtain true profiles.

CALIBRATION AND VERIFICATION OF ACCURACY

In order to obtain absolute temperatures, the instrument was calibrated against a copper-constantan thermocouple made of Leeds-Northrup thermocouple wire. This thermocouple in turn had been calibrated against a platinum-resistance thermometer which previously had been calibrated by the U. S. Bureau of Standards.

The results of this calibration for one of the thermistors are illustrated in Fig. 6. The curves are for ranges 0–50° C, 0–10° C, 9–19° C, 18–28° C, and 27–37° C. The "dots" indicate individual calibrations.

In order to be certain that the instrument will actually measure *in situ* temperatures, the instrument was compared against standard deep-sea reversing thermometers calibrated to an accuracy of ± 0.02° C. These thermometers have been used for many years by oceanographers to obtain accurate sea temperatures and their accuracy has been verified many times (Sverdrup, 1946: 347). In addition, comparisons were made against the bathythermograph (Spilhaus, 1938). Comparisons were made in sea water off the Coronado Islands, and in fresh water at Lake Hefner, a storage reservoir for Oklahoma City, Oklahoma. The thermistor, bathythermograph, and reversing thermometers were lowered in such a manner as to obtain simultaneous readings. For 23 individual comparisons with reversing thermometers, using the 10° C range scales on the TPR, the range of variation of the temperature was from − 0.1° C to + 0.2° C; 11 comparisons showed exact agreement and 10 showed a variation of ± 0.1° C. Comparisons with the bathythermograph gave a variation from − 0.3° C to + 0.2° C in 23 cases. Eighteen of the above comparisons were within a ± 0.1° C interval. From this evidence it may be concluded that the instrument has an accuracy of ± 0.1° C on the 10° C range scales.

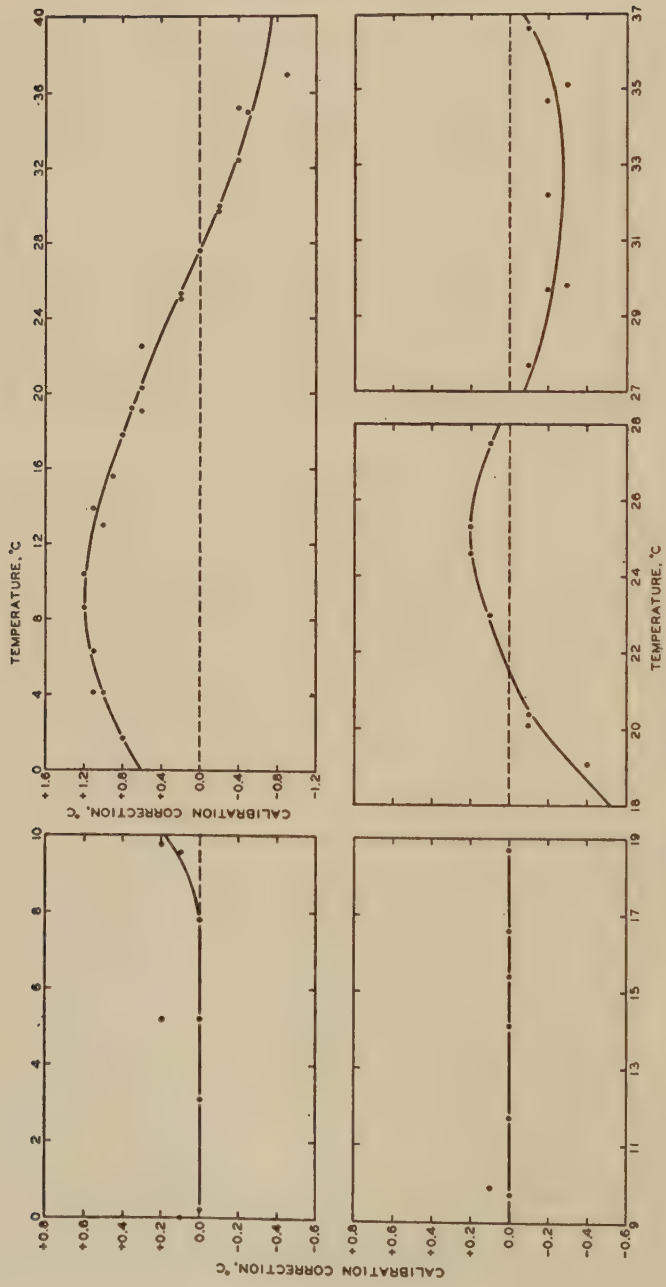

Figure 6. Calibration curves for a single thermistor.

APPLICATION

The instrument was originally developed for use in the Lake Hefner Evaporation Studies being conducted in Oklahoma (Anderson, et al., 1950). It was necessary, in this study, to obtain temperature profiles for evaluating the energy storage term in the energy budget equation. Since the average depth of Lake Hefner is approximately 10 m, it was

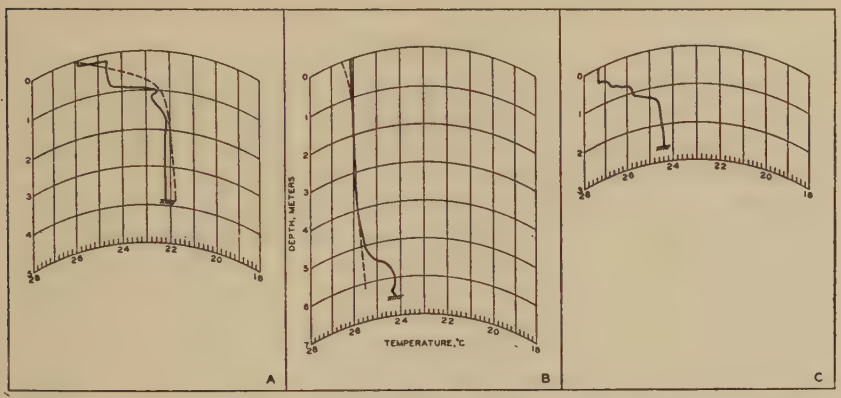

Figure 7. Temperature profiles from Lake Hefner, Oklahoma City, Oklahoma, showing comparison of a TPR (solid line) with a BT (dashed line).

necessary to obtain some of the temperature profiles in water approximately 3 m in depth. It was felt that the bathythermograph, which has been utilized previously in studies of this type, would be neither accurate nor sensitive enough when used in such shallow water.

Figs. 7A, B, and C illustrate some actual temperature profiles taken at Lake Hefner in shallow water. All three examples illustrate the detail that can be obtained by the instrument in depth intervals of only 1 or 2 m.

The profile illustrated by Fig. 7A was taken during an afternoon when the surface water was heated under relatively calm conditions and then wind-stirred to a depth of 0.8 m. The wind subsequently ceased and the upper 0.2 m (approximately 7 inches) was heated another 1.2° C. Fig. 7B illustrates a case where wind stirring had occurred to within a meter of the bottom and clearly shows the temperature distribution in the colder unmixed bottom water. Fig. 7C shows the step-like temperature profile present under heating conditions with wind stirring occurring intermittently.

The dashed lines on Figs. 7A and B indicate the bathythermograph taken simultaneously with the TPR profile.[1] From a study of Figs. 7A and B it is evident that the thermistor-sensitive instrument gives considerably more detail than the bathythermograph, especially in the top or bottom 1 m layer. In the case of Fig. 7C, it was not possible to obtain a useful bathythermogram, apparently due to the shallowness of the water.

CONCLUSIONS

1. Tests indicate that the instrument is rugged and durable, gives an easily readable record, and is capable of optimum accuracy when used in lakes, bays, or other enclosed or semi-enclosed bodies of water where temperature profiles in the upper 30 m are desired.
2. Due to the character of the instrument lag, its use should be restricted to areas where wind-wave and swell is less than 0.5 m.
3. Since the depth is recorded by measuring the amount of paid-out cable, its use is restricted to conditions of virtually zero wire angle.
4. In water, the response factor of the Western Electric (type 14B) thermistors used is 0.8 second, while the response factor for the composite instrument (thermistor, amplifier, and recorder) is 1.4 seconds. In actual use the thermistor must be lowered at a rate compatible with the strength of the thermocline in order to obtain *in situ* temperatures.
5. Comparison of the instrument, under field conditions, with deep-sea reversing thermometers and bathythermographs gives an accuracy of $\pm$ 0.1 C on the 10° C range scales.

ACKNOWLEDGMENTS

The assistance of G. W. Marks in the development of the electronic portion of the instrument, B. S. Nordahl in the development of the mechanical portion of the instrument, L. J. Anderson in the calibration and response factor determinations, and G. L. Prible in drafting the figures is gratefully acknowledged.

[1] The two profiles need not coincide, since a calibration correction must be applied to the TPR temperatures to obtain *in situ* temperatures. However, the shape of the profiles should be the same, since the variation in the calibration correction is small compared to the total range of temperature.

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AN INEXPENSIVE AND RAPID TECHNIQUE FOR OBTAINING CURRENT PROFILES IN ESTUARINE WATERS[1,2]

By

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ABSTRACT

A current indicator, consisting of a confined submerged biplane-shaped drag and a device for reading the angle made by the suspending wire with the vertical, is introduced in theory and practice. It was designed for rapid determination of current velocities and directions at any depth from a vessel anchored in shallow water. A number of calibration runs made with the Drag and with a von Arx Current Meter indicate that the Drag is reliable and sufficiently accurate for the purpose for which it was designed. The gear proved to be rugged, easy to use, and inexpensive. It requires little time to make a number of observations from the surface down to 50 feet.

INTRODUCTION

A knowledge of the circulation is of prime importance in the study of the physical characteristics of estuaries. The circulation may be inferred indirectly from the distribution of chemical constituents, or perhaps from the distribution of suspended particles. It may be determined directly from a sufficient number of current velocity measurements, which must be made at various depths in order to arrive at vertical profiles of velocity. Also, they must extend over at least one tidal cycle at any location in order to take into account the variation with tide.

Since the Ekman current meter first became the standard oceanographic instrument for current measurements, numerous attempts have been made to produce instruments superior to that in operation. The majority of these attempts has led to the construction of only a few instruments of each design and to their use by only a few investigators. The Price meter, used widely in stream work, has found some application in lakes, estuaries, and the ocean. However, the inaccuracies inherent in a design of the Price meter type in relation to the roll of the

[1] Contribution No. 2 from the Chesapeake Bay Institute.

[2] Results of work carried out for the Office of Naval Research of the Navy Department, the State of Maryland (Department of Research and Education), and the Commonwealth of Virginia (Virginia Fisheries Laboratories), under contract with The Johns Hopkins University.

boat or ship from which it is suspended are readily demonstrable (O'Brien and Folson, 1948). In addition to this, the Price type meter does not obtain subsurface current directions. Certain meters designed in Europe, notably the Ott current meters, have been used fairly extensively by European investigators. Americans, however, have used few of these.

Recently we were faced with the problem of outfitting several small vessels with means for measuring current speed and direction at surface and subsurface depths. The craft were to be used on an intensive study of an estuarine region involving relatively large tidal currents (up to two knots) and relatively shallow depths (50 feet and less). The work had to be carried out on a limited budget. Ekman current meters were not used because of their high cost and because the instrument must be brought to the surface for reading and resetting after each mesaurement.

William S. von Arx of the Woods Hole Oceanographic Institution has developed an excellent current meter which allows current speed and direction to be read on dials aboard the vessel; also, he has described a procedure for eliminating the effect of the vessel's swing on her anchor (von Arx, 1950). However, we were unable to obtain the necessary four instruments of this type within the time and budget allotted us.

Our requirements were: Availability, low cost, simplicity of operation, rapidity in obtaining measurements at all depths down to 50 feet, and dependability when used under tough field conditions. We did not feel that any commercially manufactured meter met all of these requirements satisfactorily.

One technique which met certain of our requirements involved the use of a free-drifting current drag. A current drag, or kite, with slight negative buoyancy is attached by a line to a buoy, the positive buoyance of which is just enough to support the drag. The line between the buoy and the drag is adjusted so that the drag is at the depth at which current measurements are to be made. The drag is made sufficiently larger than the buoy so that the observed movement of the buoy will reflect primarily the flow of water at the depth of the drag with but slight effect due to the action of surface current on the buoy. From the distance and direction of drift over a given time interval, the current velocity at a given depth can be determined. This technique is particularly valuable for very low velocities.

Although the free-drifting drag met certain of our requirements, we decided against its use in our work because of the difficulty in measuring accurately the distance of drift during a given time interval and because measurements at various depths require that the gear be

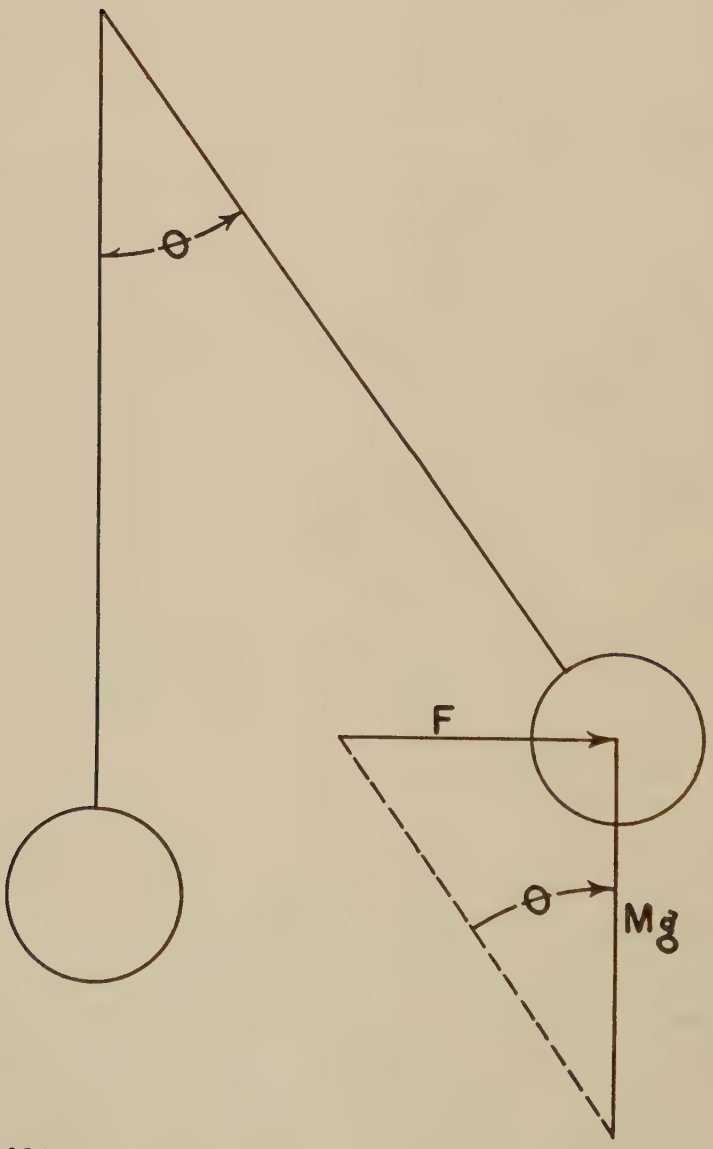

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Figure 1. The balance between the frictional drag, F, of water flowing past a confined submerged sphere and the restoring force of gravity, Mg.

recovered and that the length of the line be changed between drag and buoy. Another disadvantage is the possibility of fouling when working in fish trap areas or near the bottom of a narrow channel.

However, the technique which we finally decided to try does make use of the submerged drag, but instead of letting the drag drift freely, it is attached directly to the hydrographic wire, without a buoy; the force of the water motion on the drag is then obtained by measurement of the wire angle at the surface.

THEORY OF THE CONFINED SUBMERGED DRAG

Consider a submerged body with a negative buoyancy suspended as a pendulum in the water. This is illustrated in Fig. 1 as a sphere, but the theory also holds for bodies of other shapes. A flow of water past the submerged body will result in a force on the body, the magnitude of which is given by the expression

$$F = C_D A \rho v_0^2/2 , \tag{1}$$

where C_D is the coefficient of drag, A the cross-sectional area of the body taken in a plane perpendicular to the flow direction, ρ is the density of the fluid, and v_0 is the velocity of the free fluid (Rouse, 1946).

This force will cause the body to swing out in the direction of flow until a vector balance is reached between this force and the restoring force of gravity. From Fig. 1 it can be seen that

$$\tan \theta = F/Mg = C_D A \rho v_0^2/2Mg , \tag{2}$$

where M is the mass of the body and g is the force of gravity per unit mass of the body. Solving for v_0, the velocity of the free fluid, we have

$$v_0 = \sqrt{2Mg/C_D A \rho}\,\sqrt{\tan \theta} = k\sqrt{\tan \theta} . \tag{3}$$

Thus it is seen that the velocity of the fluid is proportional to the square-root of the tangent of the angle made by the supporting line with the vertical. The proportionality factor, k, given by

$$k = \sqrt{2Mg/C_D A \rho} , \tag{4}$$

can readily be computed for any particular drag, provided the coefficient of drag, C_D, is known.

The coefficient of drag is a function of the Reynolds number, R, and of the shape of the submerged body. Its value has been determined experimentally for a great variety of body shapes. For all bodies, C_D decreases rapidly with increasing Reynolds number until this latter parameter reaches a value between 10^2 and 10^3. For a sphere or a cylinder, C_D varies only slightly for Reynolds numbers

between 10^3 and 3×10^5. Near the higher value there is a sharp drop in C_D with increasing R due to a change in the character of the surface turbulance.

In the case of a flat plate or of a body composed of flat plates, such as the biplane shown in Fig. 2, the drag coefficient becomes independent of the Reynolds number for values of R greater than 10^3. Because of this property, together with the ease in construction, this type of drag, rather than the sphere or cylinder, was chosen for our study.

METHOD OF USING SUBMERGED DRAG

In practice, the drag, together with an added weight, is attached to the end of the hydrographic wire. It is desirable to have the meter wheel or block through which the wire runs as far out from the anchored ship as possible. At each desired depth the wire angle is determined, as is the angle between the ship's heading and the plane made by the wire and the vertical. We have prepared tables that give the required amount of wire to be let out so that the drag will be at the desired depth for a given angle. By successive adjustments, taking but a few seconds, each reading can be made at the exact desired depth.

The current speed is computed from the wire angle with the vertical, and the direction of the current is determined from the heading of the boat and the direction of the plane of the wire. Fig. 3 shows the procedure employed in measuring the wire angle.

The validity of these measurements depends primarily on three factors. First, the force on the drag must be large compared to the force of the current on the wire; secondly, the wire must remain nearly a straight line; and thirdly, the coefficient of drag must be constant over the range of flow studied. Actually, the technique would be usable if only the first factor held, for it is reasonable to assume that for any given depth the observed angle of the wire at the surface would remain a function of the current speed, even though the wire curved between the surface and the drag or if the drag coefficient changed with speed. In this case, however, the drag would have to be calibrated for each depth, and a constant proportionality factor between the current speed and the tan θ would not exist.

CALIBRATION AND NUMERICAL RESULTS

One-half inch five-ply fir plywood was used to make the biplane (Fig. 2). The dimensions on the faces of the large working models were 2 x 3 feet. A thirty-pound weight was added to the bottom. In order to make simultaneous measurements along with the drag, we

Figure 2. The current drag with weight, before lowering.

Figure 3. Reading the wire angle of the current drag. (In practice, the drag is just submerged for the surface reading.)

used a von Arx meter of the audible type, with a click being heard in earphones on deck each time the propeller turned. The current velocity was arrived at from the number of clicks heard in a given period of time.

Measurements were made from the surface to 50 feet. All of the data fitted well into the equation v (in knots) $= k\sqrt{\tan\theta}$, with k determined empirically as 1.04. Computations of the coefficient from (4) for this type of drag yielded a k value of 1.05. The value of C_D used in these computations was taken from Rouse (1946).

The velocity range within which the drag coefficient, C_D, is constant can be determined from the area of the drag. As stated previously, C_D is a function of form and of the Reynolds number. C_D remains constant for the body shape (a combination of rectangular plates) being used here for Reynolds numbers greater than 10^3. Hence

$$R_{\min} = 10^3 = \frac{DV}{\nu} = \frac{DV}{10^{-2}}, \tag{5}$$

or C_D is constant when

$$DV > 10 \text{ cm}^2/\text{sec}. \tag{6}$$

From this it is seen that, even for drags with a minimum dimension of 10 cm, C_D is constant for flows as low as 1 cm/sec (less than .02 knot). Our smallest drag has a minimum dimension of one foot. With this drag C_D remains constant according to the above expression for all velocities greater than .007 knot, and hence it remains constant for all practical velocities.

In a tidal estuary such as the James River, where we first tried this technique, currents will vary over a tidal cycle from zero to nearly two knots at times. Experience showed that it was difficult to measure wire angles of less than 3° and of more than 45°. By use of the theoretical equations, the range of velocities covered by a given drag for angles between 3 and 45° can readily be determined. For our work, two sizes of drags were constructed, one with planes of 2 x 3 feet, another with planes of 1 x 1.5 feet. Each of these drags was used with either 15 or 30 pound weights.

TABLE I. Drag Characteristics

Drag	*Wt. in Water* (*lb.*)	*k** (*knots*)	*Velocity Range* (*knots*)	*Accuracy in angle for 0.05 knot accuracy in current*		
				at 3°	*at 10°*	*at 20°*
Large—15 lb. wt.	8¼	0.64	.15–0.64	2°	3°	4°
Large—30 lb. wt.	22	1.05	.24–1.05	1°	2°	3°
Small—15 lb. wt.	12½	1.57	.36–1.57	1°	1½°	2°
Small—30 lb. wt.	26¾	2.30	.53–2.30	½°	1°	1½°

* Computed from (4) and expressed in knots.

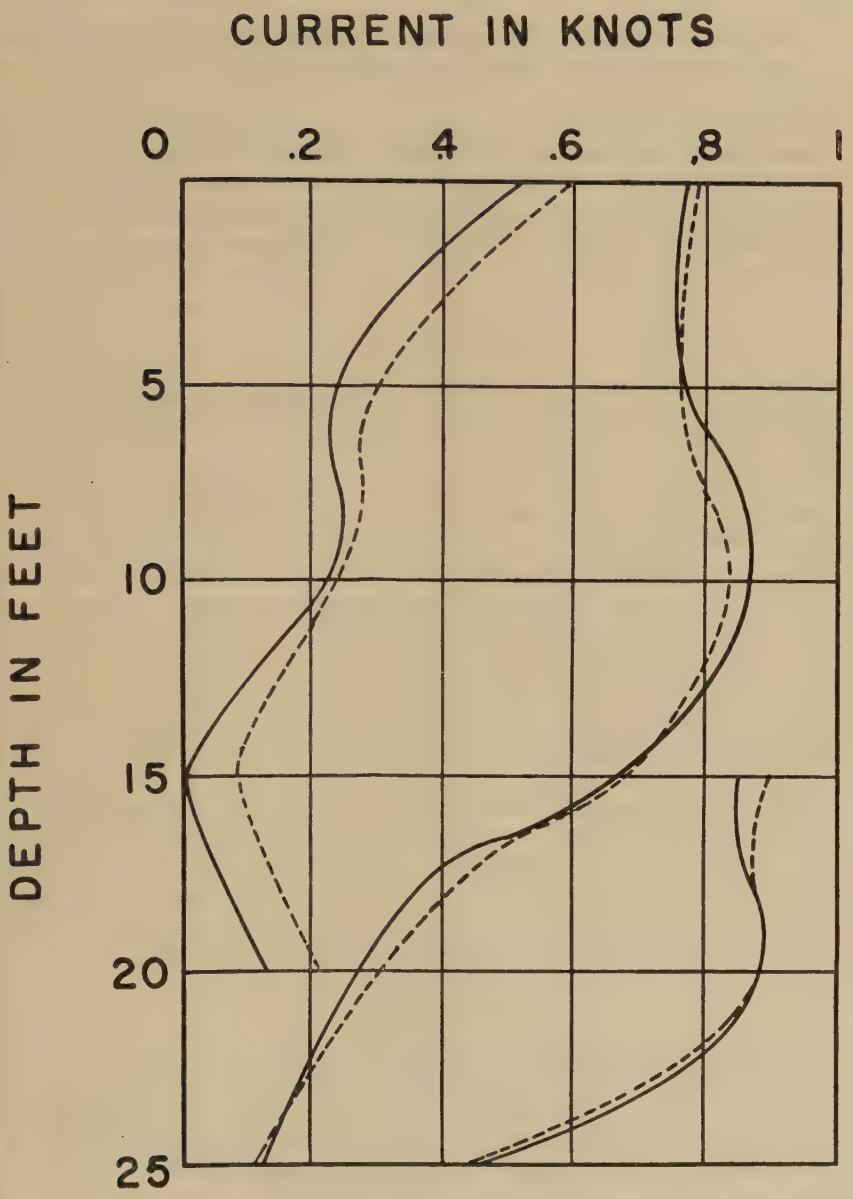

Figure 4. Comparison of velocity profile made by confined submerged drag (solid line) and the von Arx current meter (broken line).

The characteristics of these drags are given in Table I, examination of which will show that not a very great accuracy in wire angle is required for rather high accuracy in current speed.

During the course of our use of these drags, some 32 individual comparisons have been made between the velocities obtained by the von Arx meter and by the current drag for velocities of from less than 0.1 knot to 1.0 knot. The standard deviation of the readings was less than 0.06 knot. Fig. 4 shows three vertical velocity curves that compare readings from both the von Arx meter and the current drag.

THE JACOBSEN DRAG

Since this method involves well known precepts and the equipment slight expense, it appeared likely that it had been employed before, though references to its use in this country have not been found. In searching the literature, it was found that Jacobsen (1909) had proposed a similar technique early in the century. His so-called "bubble-current meter" makes use of a cylindrical drag which has several disadvantages. In the first place, a cylindrical object exhibits an unstable drag coefficient at higher Reynolds numbers. Secondly, the construction of a cylindrical drag is more difficult and more expensive than that of a biplane drag. Jacobsen presented a fine method for reading wire angle relative both to the vertical and to the ship's heading, and the interested reader is referred to his paper for details of this refinement. Since there has apparently been little or no employment of the Jacobsen meter in this country, the authors were encouraged to report the successful use of this technique.

It should be noted that we have successfully used this technique to depths of only 50 feet, though further study may show that it is suitable for observations at greater depths. Jacobsen used his meter successfully to 35 meters by correcting for wire curvature, and it is conceivable that useful current measurements from greater depths may be obtained with light wire, such as piano wire.

SUMMARY

A biplane type suspended drag can be used with accuracy to determine current direction and speed from an anchored vessel in shallow water. The gear is inexpensive (less than $15 per unit for materials at the present time), rugged, and easy to use, and it takes little time to make repeated observations at any depth down to 50 feet.

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A PLASTIC ENVELOPE SUBSTITUTE FOR DRIFT BOTTLES

By

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ABSTRACT

A plastic envelope of 0.004-inch thick polyethylene is suggested as a substitute for drift bottles. The return card is sealed hermetically in the envelope, thus forming an inexpensive, compact and convenient means for studying surface currents by conventional drift bottle technique. Tests indicate that "drift cards" actually indicate surface flows and are not at the complete mercy of the winds.

In certain investigations of lake or ocean currents, drift bottles will provide information unobtainable otherwise. This is particularly true in shallow coastal regions and in areas with many shoals and islands or with an irregular coastline. However, drift bottles are fragile and may break either in handling aboard ship, or, more likely, when washed against rocky cliffs or beaches. To use them effectively, a great number should be released on a single cruise, in which case they will require considerable storage space aboard ship. The materials and labor required in preparing large numbers of bottles are often costly.

DRIFT CARDS

In the course of an extensive study of currents in western Lake Erie (Olson, 1950), it was necessary to consider seriously the likelihood of breakage of bottles when they stranded on rocky coasts. This problem was entirely eliminated by substituting flat transparent plastic envelopes for the glass bottles. The writer has called them "drift cards" to distinguish them from drift bottles in spite of the fact that the term "drift envelope" might be more logical.

The advantages of drift cards are many. There is no problem of storage aboard ship, since 200 cards can be held in one hand. The cards float "face up," exposing no surface above the water and thus eliminating the need for ballasting. While no data on comparative costs are available, it is believed that cards will be considerably cheaper than bottles (less than one cent for materials alone).

Preparation. In a series of trials conducted by the writer, the most suitable material was found to be a transparent polyethylene sheet, 0.004 inch thick, made in the form of continuous tubing 6 inches wide

when flattened. The tubing is cut into strips 4 to 4.5 inches long; the return card is inserted into the tubing, which is then heat-sealed along the cut edges. Once the plastic is sealed, it is almost impossible to tear it with the fingers alone; therefore, the card should bear instructions to open with a sharp knife or scissors.

From examination of cards returned in good condition 18 months after liberation, it appears that the polyethylene envelope offers sufficient protection under most conditions. It was noted that cards returned from sandy beaches showed definite signs of sand abrasion; to overcome this problem, a greater thickness of plastic sheet (perhaps 0.006 to 0.008 inches) may be advisable.

Multiple Releases. Some workers with drift bottles have released but one bottle at a given point while others have released several hundred at one point. Since drift cards are cheap and easy to handle, there is little justification for the single-release method.

In the Lake Erie studies all cards were numbered serially from 1 to 1500 and a fixed procedure was adopted in releasing them. If, for example, 10 cards were to be released at one place, the 10 were withdrawn and their serial numbers checked. From a point near the stern of the vessel, the lowest numbered card was first thrown overboard and watched until it fell into the lake before the next card was thrown out. This almost trivial precaution is necessary to eliminate the possibility of the wind picking up the card and dropping it on top of the cabin or elsewhere where it might remain for an indefinite length of time before being blown into the sea eventually. This method also prevents two or more cards from sticking together in the water for an indefinite length of time.

In nearly all cases, 10 cards were released consecutively, usually within 30 seconds. This method of multiple releases provides more dependable data than would be obtained if the cards were released singly.

The value of multiple releases is well shown in the 441–450 series of the Lake Erie studies; 10 cards were released at 1147 hours on 21 July 1949 at a point three miles south and one mile east of the Detroit River Light. Of the 10 cards released, 9 were recovered, all in the immediate vicinity of Colchester, Ontario. The times of recovery are given below:

Card No.	*Date and time found*
441	27 July 1930 hrs
442	2 Aug 1530
443	2 Aug 1800
445	27 July 0700

Card No.	*Date and time found*
446	26 July 1900
447	27 July 1930
448	27 July 1930
449	5 Aug 1330
450	27 July 1930

A glance at these figures will show that the cards must have stranded during the afternoon of 26 July. The fact that five cards were also found the following day at 0700 and 1930 hrs is indicative that the beach was visited fairly frequently; thus the time adrift is established with reasonable accuracy.

On the same date, cards were also released at 19 other points. By comparing results from nearby points, additional confidence is given to the estimated time adrift.

Effect of Wind on Drift Cards. The fear has often been expressed that drift cards will be at the complete mercy of the winds, since they are light and float flat side up on the surface of the water. It is important that this question be resolved before the value of drift cards can be appraised properly.

In protected bays and small lakes, particularly during an algal bloom, it has been observed frequently that the surface waters will move in one direction while the water only an inch below the surface will move in a different direction. Extreme cases of this nature have not been reported in open waters of larger lakes and seas, and it is doubtful if the high turbulence found there will ever permit a micro-stratification of this nature. Obviously, if such conditions arise, the use of drift cards may be questioned.

The Lake Erie studies provide several striking examples of drift card travel that is independent of the prevailing or resultant wind. On 9 June 1949 at 1139 hrs, 20 cards (161–180) were released eight miles north and five miles east of Marblehead Light (on Catawba Peninsula). One card stranded on Cedar Point and 14 at Marblehead. On 12 June, cards were found at 1400, 1545, 1600 and 1900 hrs, establishing the time adrift as 3.1 days. The drift was 2.9 miles per day S 35° W when the resultant wind during this time was 3.5 mph due south (blowing toward the north).

Another example of the independence of drift and wind is given by the 1211 to 1281 series released on 22 August 1949 in the general vicinity of the mouth of the Detroit River. The 1211–1220 set was released at 1225 hrs and the last set, 1281–1290, was released at 1445 hrs; thus all cards were liberated during a 140 minute period. The 1211, 1221 and 1231 sets were released west of the Detroit River and *all*

drifted due west to WSW. The 1241 and 1261 sets were liberated at the Detroit River Light and all recoveries were made from Point Pelee, some 40 miles east. The 1251 set was liberated in the Detroit River. The cards were recovered in the same general neighborhood as the 1241 and 1261 sets, but they had to travel *south* in the Detroit River before swinging eastward to join the 1241 and 1261 sets. The 1271 set, released 1.5 miles south and 9.5 miles west of Colchester, all stranded at Colchester and traveled almost due east. On the other hand, the 1281 set, released about 12 miles SW of Colchester, also stranded at Colchester.

Thus, of 80 cards released within 140 minutes at eight fairly closely spaced points in a region wherein three distinct current systems originate, we found distinct drifts due west, due east, south and northeast. Similar results were obtained at other times, notably in the 421–481 series released on 21 July 1949 in the same general area. The drifts indicated by these cards are perfectly consistent with flow patterns established by other methods such as water mass analysis and the drift bottle studies of Harrington (1895). They indicate conclusively that drift cards are not at the complete mercy of the wind.

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AN AUTOMATIC REAGENT DISPENSER FOR SHIPBOARD USE[1]

By

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Recent work on modification of the molybdenum-blue method for determination of dissolved inorganic phosphate in sea water (Wooster and Rakestraw, 1951)[3] showed the need for a simple rugged device suitable for rapid and precise delivery of small quantities of reagents at sea. The two reagents used for phosphate determination impose additional requirements on this device. Molybdic acid solution must be protected from the light and must not be allowed to come in contact with rubber; stannous chloride solution must be protected from contact with the atmosphere.

An automatic reagent dispenser which meets these requirements is shown in Figs. 1 and 2. Volume of reagent delivery is governed by travel of the spring-loaded syringe plunger, which in turn is controlled by a series of carefully machined steps. Dimensions of these steps are determined by bore of the syringe plunger and by delivery volume required. The syringe assembly, acting through a length of Tygon tubing, pumps mineral oil which comes in contact with the reagent solution at an oil-reagent interface. After the delivery system of the dispenser has been loaded by withdrawal of the plunger, unit delivery of reagents is effected by stepwise release of the plunger. In order to protect reagents from light and glassware from breakage, glassware and reagent bottle are customarily enclosed in a wooden box. This box is provided with an opening into which can be inserted the sample bottle to which reagents are to be added.

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[3] Wooster, W. S. and N. W. Rakestraw, The estimation of dissolved phosphate in sea water, J. Mar. Res., *10* (1): 91–100, 1951.

Two such dispensers were calibrated by weighing deliveries of distilled water. One dispenser made 20 deliveries, using each step in succession, with an average volume of 0.205 ml and a standard deviation of 0.004 ml; the other dispenser made 20 deliveries with an average volume of 1.002 ml and a standard deviation of 0.010 ml.

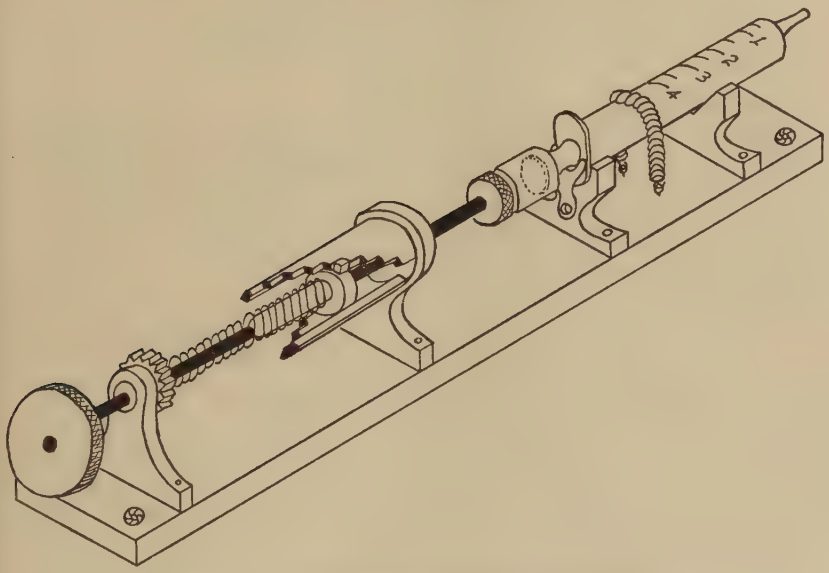

Figure 1. Syringe assembly for delivery of 0.50 ml.

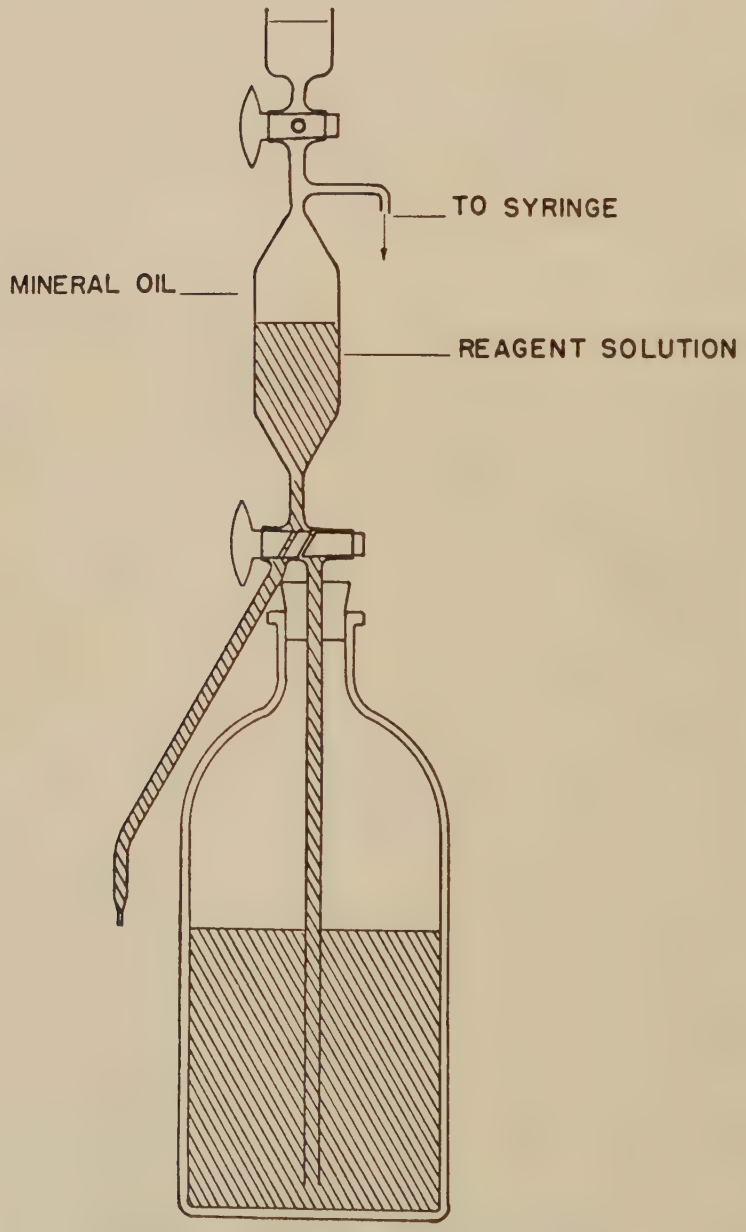

Figure 2. Glassware for automatic reagent dispenser.

DELAYING SPAWNING OF LAMELLIBRANCHS BY LOW TEMPERATURE

By

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ABSTRACT

Spawning of oysters (*Crassostrea virginica*) and clams (*Venus mercenaria*) was delayed by transferring ripe or nearly ripe animals from Long Island Sound to the cold waters of Boothbay Harbor, Maine, where the temperature, although high enough to permit gametogenesis, was too low to induce spawning. With *C. virginica*, spawning could be postponed until six or eight weeks after the Long Island Sound population was completely spent, while *V. mercenaria* held over healthy summer spawn throughout the fall and winter and into the following spring. By similar means, laboratory workers can be provided with ripe animals during the fall when none are normally available in their own locality.

Until recently the eggs and larvae of lamellibranchs, such as oysters and clams, were available only during their normal spawning period. In our waters this period is relatively short, usually from about 2½ to 3 months. However, several years ago a method was developed to induce oysters to develop gametes and to discharge spawn in winter (Loosanoff, 1945). Later the same method, in somewhat modified form, was successfully applied to other mollusks (Loosanoff and Davis, 1950).

The method merely consists of a gradual increase in the temperature of the water from the low level prevailing in winter over the mollusk beds to that at which they spawn in summer. Using this simple method, *Crassostrea virginica*, *C. gigas*, *Ostrea lurida*, *O. edulis* (the European oyster), *Venus mercenaria*, *Mya arenaria*, *Mactra solidissima*, *Anomia aculeata*, *Ensis directus*, *Petricola pholadiformis*, and several other lamellibranchs have been conditioned at our laboratory to develop and discharge normal gametes throughout the winter and spring.

In the same way we have also succeeded in ripening the common starfish of Long Island Sound, *Asterias forbesi*. The gastropod oyster drill, *Urosalpinx cinerea*, was also conditioned to deposit egg-cases in the middle of winter. We think that the method is well suited to ripen for spawning many other invertebrates, including annelids, the eggs and larvae of which were often found when we spawned oysters in winter.

Conditioning lamellibranchs for spawning in late fall and winter is possible only after they recover from the natural summer spawning. Recovery consists of an accumulation of reserve materials, such as glycogen. In our waters that stage may not be reached by some lamellibranchs until the end of October. Thus, since these lamellibranchs become spent some time during late August or early September, and since they cannot be conditioned for fall and winter spawning until November, their ripe sex products are not available during the intervening period. To solve the problem of providing the laboratory with mollusks in spawning condition during this period, the following experiments were made.

At the end of May 1950, several bushels of adult oysters (*C. virginica*) and clams (*V. mercenaria*) from Long Island Sound were planted in the waters of Boothbay Harbor, Maine. Since the water of Boothbay Harbor in summer is about 5–8° C. cooler than that of Long Island Sound, we reasoned that the temperature of the Boothbay Harbor water, while not arresting the development of the gametes of our mollusks, would, nevertheless, remain low enough to prevent the mollusks from spawning. Thus, by the time the oyster and clam populations of Long Island Sound were completely spawned out, or nearly so, those kept in the Maine water would still retain sperm or eggs and could, therefore, be induced to spawn when needed.

Oysters and clams of the first group, brought from Maine on September 7, 1950, were ripe with extremely thick gonads and were easily induced to spawn with a discharge of large quantities of eggs and sperm.

The second group was brought to Milford on October 3 nearly a month later. These oysters were ripe also, but unlike the first ones, only some of them could be induced to spawn while others already showed evidence of resorption of the unspawned ova (Fig. 1). The clams, on the other hand, showed no evidence of spawning or of resorption of gonadal material. They responded quickly to spawning stimulation, which consisted of a rapid increase in the water temperature to about 32.0° C. and the addition of egg or sperm suspension; large quantities of normal spawn were discharged.

The third group was brought from Maine on October 24, about three weeks later than the second batch. At that time the temperature of the water at Boothbay Harbor was approximately 9.0° C. After being brought to Milford, the mollusks were placed in the large tide-filled outdoor concrete tank where the temperature was about 10.0° C. An attempt to spawn the oysters of this group on October 26 was unsuccessful. Of the 40 oysters used, only one male responded, and even this was only a feeble spawning, consisting of a discharge of sperm with considerable quantities of amorphous matter and blood

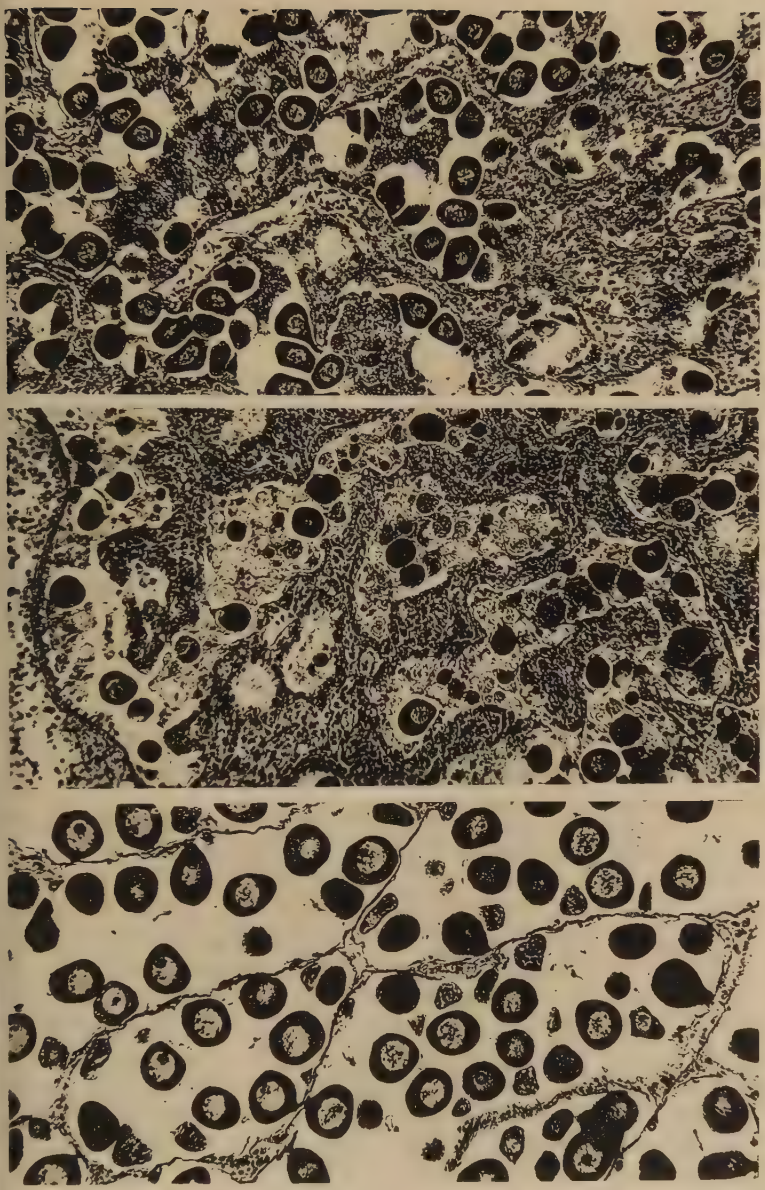

Figure 1. Gonad of female oyster in early stages of resorption. X112 Figure 2. Gonad of female oyster in advanced stages of resorption. Notice many cytolized eggs. X112 Figure 3. Section of gonad of experimental clam, *Venus mercenaria,* preserved in December. X112

cells. Examination of the remaining 39 oysters showed that resorption was already in progress in the majority of them and was well advanced in some (Fig. 2).

The clams that were brought to Milford on October 24 were distinctly different from the oysters in both condition and behavior. There was no evidence of resorption of gonadal material, and two days after arrival every one of the 15 clams responded to spawning stimuli. The discharged eggs developed into larvae which were cultured to metamorphosis.

Because the oysters were resorbing their gonads by the end of October, no further attempts were made to spawn them. However, systematic observations on the clams were continued, since their condition and their physiological behavior were different. The source of supply for these observations was the large group of clams that was brought from Maine on October 24, 1950; these were kept in our outdoor tank. A sample consisting of 10 individuals was taken from the tank on November 7 and again on November 15. All of the clams spawned in response to stimulation. In the latter case the clams came directly from water of only 8.1° C. The eggs obtained from both spawnings developed normally and the larvae were again cultured to the setting stage.

As the season progressed, groups of clams were brought into the laboratory from the outdoor tank at approximately biweekly intervals to ascertain whether they still could be induced to spawn and produce viable zygotes. It was noticed, however, that the clams did not respond to spawning stimuli as readily as they did earlier in the season. Sometimes they had to be kept at high temperatures for 10 or 12 hours before responding. Yet, histological examination of specimens killed and preserved immediately upon their removal from the tank showed that their gonads contained normal eggs (Fig. 3). Apparently the delay in the spawning response was due to keeping the clams in the tank at a low temperature, which was down to 6.0° C. by the beginning of December. From then on, however, the clams were brought to the laboratory and were kept there for a day or two in running water of approximately 20.0° C. before being subjected to spawning stimuli. As a rule the clams responded favorably to such treatment and, upon stimulation, discharged large quantities of spawn.

The last group of clams was induced to spawn on April 9, 1951; the larvae received from this spawning were grown to metamorphosis. Unfortunately, the sample of April 9 exhausted our supply and observations had to be discontinued.

In general, the experiments showed that it is possible to delay spawning of ripe or nearly ripe mollusks by keeping them at a com-

paratively low temperature. In oysters it can be delayed as late as mid-October. In other words, under natural conditions most of the oysters in our waters would be spent in August, but by using this method we can delay spawning for 6 or 8 weeks, i. e., until the time of year when the oysters living in Long Island Sound will be ready to be conditioned for winter spawning. Spawning of *V. mercenaria* can be delayed even longer than that of oysters. Thus, using a combination of our two methods, one of conditioning for winter spawning (Loosanoff and Davis, 1950) and the other of delaying spawning in late summer and fall by low temperature, it is possible now to have ripe lamellibranchs available throughout the year.

Somewhat similar results in the delay of spawning were achieved by taking ripe mollusks from Long Island Sound in midsummer and by keeping them in the laboratory in an insulated container through which mechanically-cooled sea water was flowing. Basically the principle is the same as keeping them in the cool water of Maine. However, only a small number could be conveniently kept in the laboratory under semirefrigerated conditions. Furthermore, a rapid increase in temperature due to failure of the mechanical devices always caused major and complete spawning, thus rendering the whole group useless.

Although observations on the fate of the unspawned ova and sperm of clams and oysters are not the subject of this article, it should be mentioned here that the ability of the clams to carry undischarged spawn far into the winter, without undergoing such cytological changes that would make the gametes unviable, is of extreme biological importance and interest. It is even more significant that the retention of healthy spawn through late winter and spring occurred although the clams were exposed for several months to temperatures ranging between 0 and 6° C.

Perhaps this ability of the clam, unlike that of the oyster, which resorbs its gonads early in the fall, can be explained by the general pattern of its seasonal gonadal changes (Loosanoff, 1937). According to this pattern the production of a new crop of eggs in *Venus mercenaria* begins soon after spawning, and the chief growing period of young ovocytes is in autumn during October and November. By the onset of winter the gonads of female clams usually present a virtually mature appearance. The cells developed in autumn remain in the clam gonads throughout the winter without undergoing any degenerative processes. Nevertheless, regardless of this consideration, we should not underestimate the biological significance of the observation that normal eggs and spermatozoa which were discharged by the clams on April 9, 1951 were those which were formed some time in October or November of 1949, i. e., almost two years ago.

We are deeply indebted to our colleagues, John B. Glud and Walter R. Welsch of the U. S. Fish and Wildlife Service biological laboratory at Boothbay Harbor, Maine, whose help and friendly cooperation enabled us to carry on this study, to Charles A. Nomejko for preparing the photographs for this article, and to William S. Miller for his help in taking the mollusks to Boothbay Harbor.

SUMMARY

1. It was possible to delay spawning of clams and oysters for considerable periods by keeping them in water, the temperature of which was high enough to permit gametogenesis but too low to induce spawning.

2. While the oyster, *C. virginica*, resorbs unspawned gonads in late fall, the clam, *V. mercenaria*, carries undischarged eggs or spermatozoa through the winter and even into spring; the latter may be induced to spawn during this time, discharging viable gametes which develop into normal larvae.

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THE SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE IN SEA WATER[1]

By

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ABSTRACT

The zirconium-alizarin sulfonate indicator of Lamar has been applied to the spectrophotometric determination of fluoride in sea water and has been shown to be suitable. Calibration curves have been prepared for different chlorinities. The variation of apparent fluoride content with chlorinity is less than that reported by Thompson and Taylor with their reagent. Borate, iron and phosphate have been shown not to interfere at the concentrations found in sea water, while a method has been given to take care of varying alkalinities. The effects of time, temperature and light upon the color intensity have been discussed. Increased accuracy is attained through the measurement of the color intensity spectrophotometrically.

INTRODUCTION

The fluoride content of sea water has been determined colorimetrically by a method developed by Thompson and Taylor (6) in which the reagent is a reddish zirconium-alizarin sulfonate lake formed by the reaction of zirconium nitrate and alizarin sulfonate. Fluoride, in the presence of acid, reacts with and destroys this reddish lake, forming colorless zirconium fluoride and yellow alizarin sulfonate. The fluoride content is determined by measuring this bleaching of the lake.

Since the work of Thompson and Taylor (6) in 1933, no work on the zirconium-alizarin sulfonate method of determining the fluoride content in sea water has been reported in the literature, though many modifications of the method have been reported for fresh waters, starting with that of Sanchis (4). Lamar (2), in his work on fresh waters, used a reagent containing sulfuric acid to lessen the interference of sulfate in the water; this is one of the most active interfering substances in the fluoride determination. In ocean waters there is considerable sulfate and in coastal waters its concentration varies widely, thus making difficult the compensation for its interference. It was thought that use of Lamar's indicator might well reduce the effect of sulfate in marine waters. The study of the use of this indi-

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cator was done spectrophotometrically to get greater accuracy and to eliminate the necessity for the preparation of reference standards, as is the case when using Nessler tubes. The results of this investigation are reported in this paper.

SOLUTIONS AND CHEMICALS

All chemicals used in this investigation were C. P. Grade.

Indicator:

(A) 1.840 g of $ZrO(NO_3)_2 \cdot 2H_2O$ were made to 250 ml with distilled water and filtered.

(B) 0.370 g of alizarin red S, $C_6H_4(CO)_2C_6H_4(OH)_2SO_3Na$, were dissolved in 250 ml of 2.10 N H_2SO_4.

Twenty-five ml of solution A were diluted with 100 ml of distilled water, 25.00 ml of solution B were added with stirring, followed by 500 ml of 2.10 N H_2SO_4 and sufficient distilled water to make a liter. This reagent was permitted to age 24 hours before using.

Standard Fluoride Solutions. A stock solution containing 100 μg (0.100 mg) F^-/ml was prepared by dissolving 222 mg of pure NaF (dried at 105° C) in water and diluting to a liter. A solution containing 10 μg F^-/ml was prepared by ten-fold dilution with water.

Dilute Standard Fluoride Solutions. Fluoride solutions ranging from 0 to 1.5 mg F^-/L were prepared in a synthetic sea water medium of chlorinity 17 °/₀₀ unless otherwise specified. This is the chlorinity approximating the waters of Puget Sound and the Strait of Juan de Fuca. Synthetic media of this concentration were prepared from stock solutions made from NaCl and $MgSO_4 \cdot 7H_2O$. The sodium chloride solution was made to contain 160 g/L and the magnesium sulfate solution 34.9 g $MgSO_4 \cdot 7H_2O$ per liter. These concentrations were verified through analysis for Cl^- and $SO_4^=$. One hundred ml of each of these solutions, when mixed and diluted to 500 ml with distilled water, gave a chloride and sulfate concentration corresponding to a sea water of chlorinity 19 °/₀₀; or, 89.47 ml of each of these solutions, when mixed and diluted to 500 ml with water, gave a chloride and sulfate concentration corresponding to a chlorinity of 17 °/₀₀.

METHOD OF ANALYSIS

Sea water samples of 50 ml volume were measured into 125 ml Erlenmeyer flasks. The alkalinity of each water sample was neutralized to pH 4 through the addition of a volume of acid which had been determined by acid titration of a separate 50 ml volume of sea water, using an appropriate acid-base indicator. Adjustment of the pH of the sample could also be done through acid titration, using a glass

electrode as indicator. Five ml of the reagent solution were added and the mixed solution was maintained at 28° C in a thermostat for six hours. The optical density of the solution was read in a Beckman DU spectrophotometer at a wavelength of 512 mμ using a 10 cm cell.

The optical density readings in this paper are reported in respect to distilled water reference solutions. Since greater accuracy could be attained in the lower range of transmissions by using a reference standard approximating the unknowns in optical density, a solution of cobaltous nitrate with an optical density of 0.242 was used for this purpose. Such a value is close to that given by the indicator in a solution of 17 °/₀₀ chlorinity containing no fluoride. For routine analysis of sea water, a solution of optical density 0.140, near that obtained for 0.9 mg F^-/L, would be more convenient and accurate.

EXPERIMENTAL

Optical Density Curves at Various Wave Lengths. Absorption at various wavelengths was measured on synthetic sea water samples, the results of which are shown in Fig. 1. Maxima were observed in

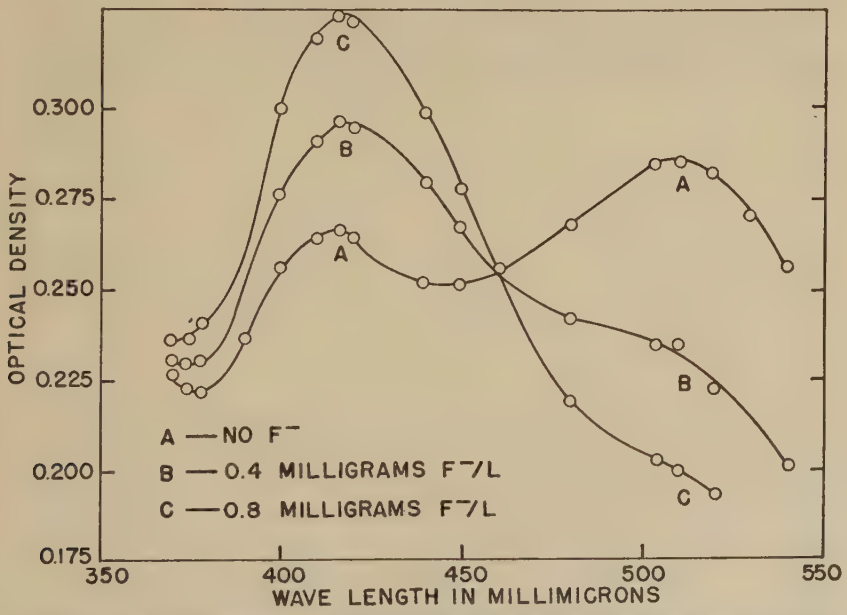
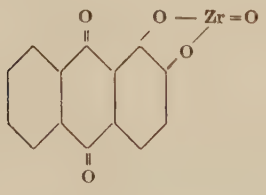


Figure 1. Absorption spectra of zirconium-alizarin sulfonate lakes in synthetic sea water solution, Cl = 17 °/₀₀. Curve A: Absorption spectra of lake with no fluoride present; Curve B: Absorption spectra of lake with 0.4 mg F^-/L present; Curve C: Absorption spectra of lake with 0.8 mg F^-/L present.

the curves at 418 and 512 mμ. The former is due to free alizarin sulfonate and the latter to the zirconium-alizarin sulfonate lake. With the addition of fluoride, the absorption at 418 mμ increased because of the displacement and liberation of alizarin sulfonate from the lake by the fluoride, while at 512 mμ the absorption decreased because of the destruction of the lake by the reaction of the fluoride with the zirconium. With sufficient fluoride the maximum at 512 mμ disappeared. It is quite possible to estimate the fluoride content of the sample at either wavelength, and in the exploratory work the readings were taken at both wavelengths. Interfering substances have the same relative effect at either wavelength. Eventually the 512 mμ wavelength was selected as the more desirable for the analysis because of the greater sensitivity to fluoride concentration at this wavelength.

Composition of the Zirconium-Alizarin Sulfonate Reagent. The composition and structure of the zirconium-alizarin red S reagent is not at all certain. From a spectrophotometric study of zirconium-alizarin lakes, Flagg, Liebhafsky and Winslow (3) concluded that the lakes are definite compounds, formed with a molar ratio of zirconium/alizarin of 1/1 and with the structure shown in formula I, regardless of whether they were formed from equivalent amounts of zirconium and alizarin or with one in excess. By analogy the zirconium-alizarin red S lake would have the formula shown in formula II.



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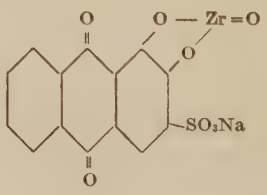

II

On the other hand, Weiser and Porter (7), from their investigations, decided that lakes are not definite compounds at all but rather are held together by adsorption forces.

The zirconium-alizarin red S lakes to be used for fluoride reagents are prepared with the zirconium in large excess. In Lamar's reagent, zirconium is in excess, with a zirconium-alizarin red S ratio of 6.3. Since this reagent is prepared in a 1.1 N sulfuric acid solution, most of the zirconium excess would be complexed with sulfate ion forming $ZrSO_4^{++}$, $Zr(SO_4)_2$, and $Zr(SO_4)_3^{=}$ (1).

The possibility of improving the sensitivity of the Lamar reagent

through change in composition led to experiments with varying ratios of zirconium/alizarin sulfonate. It was determined that the addition of 25% extra $ZrO(NO_3)_2$ to Lamar's indicator lessened the sensitivity of the indicator to fluoride and resulted in greater fluctuations with sulfate. Addition of 25% extra alizarin red S to the normal indicator gave slightly greater sensitivity to fluoride at moderate sulfate concentrations, but this resulted in considerable loss of linearity

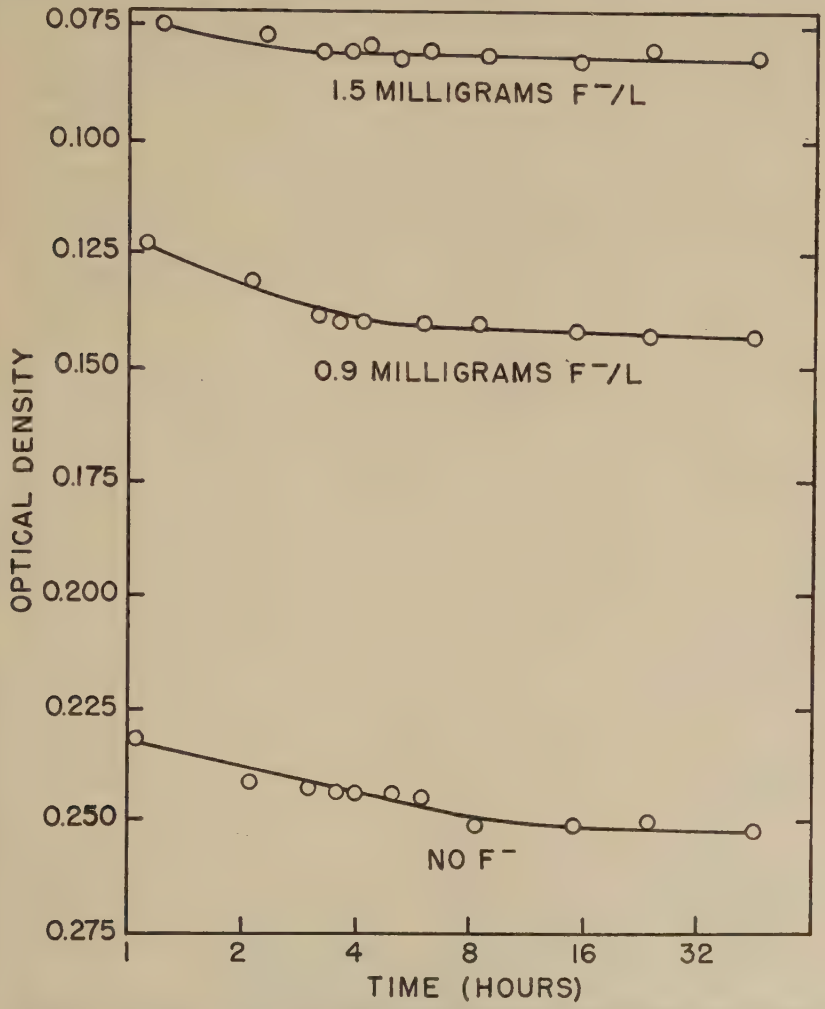

Figure 2. Effect of time upon the color development in synthetic sea water solution, Cl = 17 °/₀₀. Optical density observations at 512 mμ.

of fluoride response. Consequently Lamar's reagent was used unchanged in composition.

Effect of Time on Color Development. The development of color with time is shown in Figs. 2 and 3 for the wavelengths 420 and 512 mμ. It can be seen that no constant value is reached within any reasonable time. However, the variation after 4 or 5 hours is slight, and a 6-hour time for color development was selected as satisfactory from a standpoint of stability and convenience. A time variation of plus or minus an hour gave an error considerably less than the random error of the determination. At considerably greater times, such as 27 or 48 hours, there was loss in precision, so that longer times seemed undesirable.

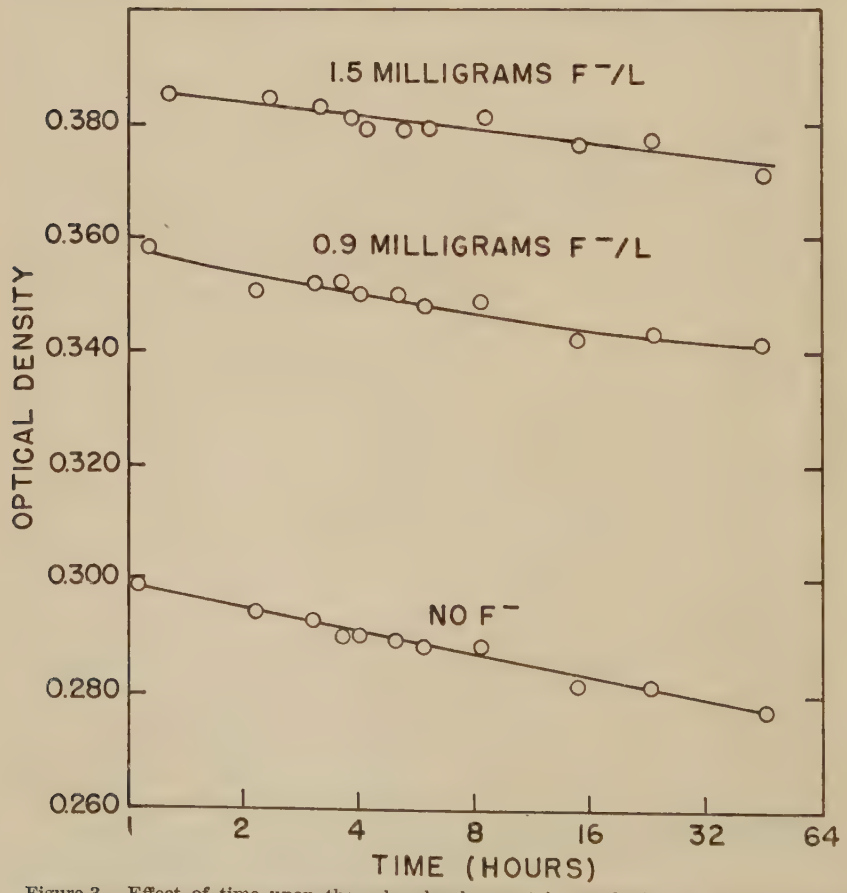

Figure 3. Effect of time upon the color development in synthetic sea water solution, Cl = 17 °/₀₀. Optical density observations at 420 mμ.

The time of aging the indicator before its use is likewise important. Lamar recommended that the indicator stand at least an hour after its preparation before being used to insure equal development of standards and unknowns. Spectrophotometrically, since there are no comparison standards to compensate for changing sensitivity of the reagent, it is necessary to have an indicator sensitivity that is constant with time. It was found that spectrophotometrically the indicator attained a satisfactory constancy after an aging period of 24 hours.

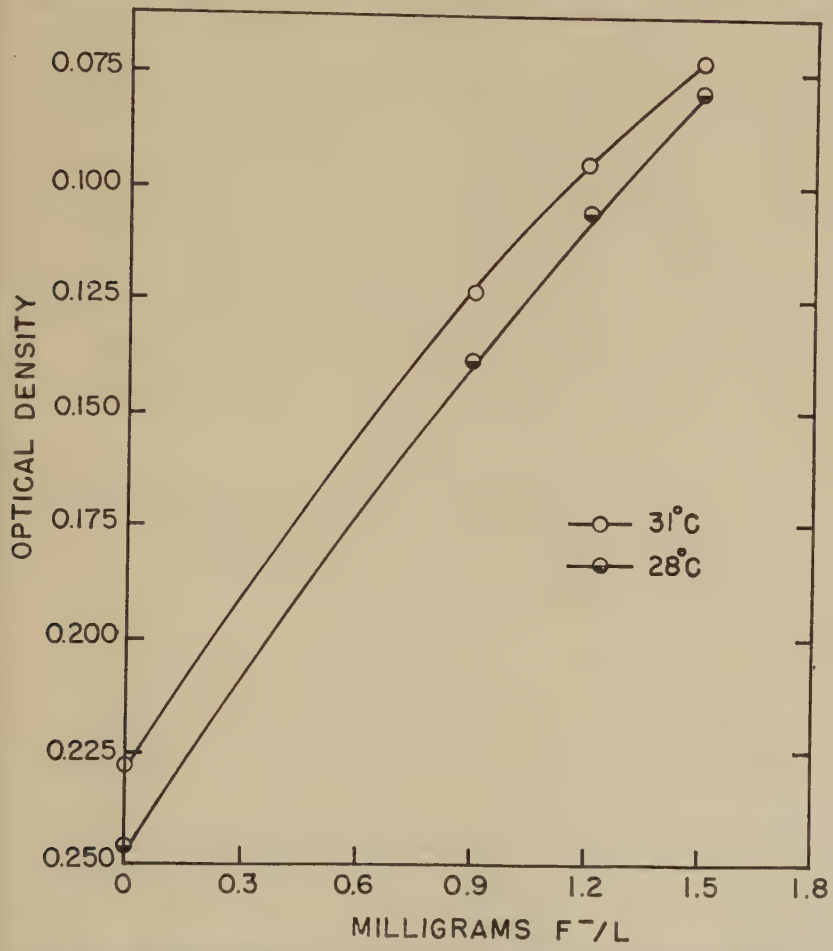

Figure 4. Effect of temperature upon the color development at various fluoride concentrations. Synthetic sea water solution, Cl = 17 °/₀₀. Optical density observations at 512 mμ.

Freshly prepared indicator gave less lake color, which is interpreted as an apparent fluoride content higher than the true value. After the 24-hour aging period, the sensitivity changed but slowly, giving a result only about 1% low even after a month's standing under room conditions (in the dark).

Effect of Temperature on Color Development. Variation in temperature greatly changed the optical densities involved in this determination. The incubation temperature of 28° C for this work was selected because it was a convenient temperature slightly above room temperature. Fig. 4 illustrates the effect of a small increase in the incubation temperature. Reproducibility then demands a constancy in incubation temperature and sufficient rapidity in making the spectrophotometric measurements so that no appreciable temperature change takes place either by exchange of heat with the surroundings or by absorption of energy from the light passing through the sample.

Effect of Light on Color Intensity. From preliminary investigations it was thought that illumination during incubation changed the optical densities. This was disproved when it was found that the optical densities of samples exposed to 13 to 14 foot-candles of indirect illumination for the six-hour incubation period were unaffected. Of course, change in temperature due to absorption of light during incubation or measurement of the optical densities would affect the results, as mentioned previously.

Effect of Volume of Solution on Color Intensity. Experimentally it was found that 300 ml samples, for example, did not develop exactly the same optical density as 50 ml samples. This, possibly, was a surface effect, but it was not investigated at any length. It was avoided by taking the same volume and the same size flasks for all samples.

Effect of Hydrogen-ion Concentration on Color Intensity. Hydrogen ion has considerable effect on the optical densities observed, the effect being similar to that given by fluoride. For this reason the acid content of the indicator should be maintained at a constant level and the hydrogen-ion concentration of the samples should be adjusted. In determining this effect, samples of varying fluoride content were made 0.01 N in respect to hydrogen ion by the addition of 0.5 milliequivalent H_2SO_4 to 50 ml of solution, and these were compared with a series of samples to which had been added an equivalent amount of magnesium sulfate to compensate for the sulfate effect. It can be concluded from these results, as shown in Fig. 5, that this concentration of hydrogen ion gave an apparent increase of 0.21 mg F^-/L at a level of 1.2 mg F^-/L. An identical experiment with HCl and NaCl gave essentially the same results.

Present in sea water are a number of anions of weak acids which react with hydrogen ion and buffer the solution. The ones in largest quantities are HCO_3^-, $CO_3^=$ and $H_2BO_3^-$. The effect of these ions on the determination of fluoride is illustrated by the following experiments with HCO_3^-. Sodium bicarbonate was added to a series of

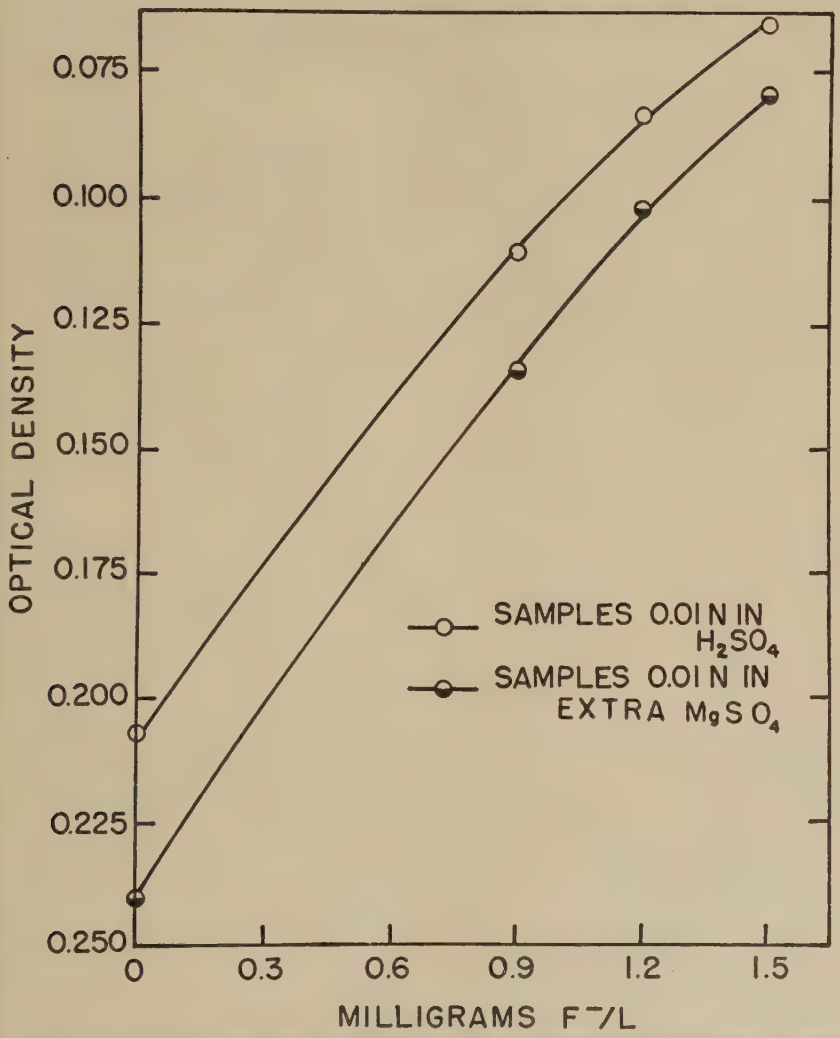

Figure 5. Effect of H^+ and $SO_4^=$ upon the color development of various fluoride concentrations. Synthetic sea water solution, Cl = 17 °/₀₀. Optical density observations at 512 mμ.

samples to give a concentration of 250 mg HCO_3^-/L, which is equal to twice the amount expected in sea water of Cl = 17°/₀₀ (5). The fluoride readings at 0.9 and 1.2 mg F^-/L were low by 0.13 mg and at 1.5 mg F^-/L they were low by 0.21 mg/L.

These results would indicate that the effect of the hydrogen ion is neither constant over a range of fluoride values nor linear with respect to the amount of acid added. Accordingly a good procedure for the determination of fluoride would avoid changes in acidity rather than attempt to apply a correction factor.

Effect of Sulfate Interference. Using Fig. 5, the effect of sulfate may also be determined by a comparison of the magnesium sulfate curve with a curve for a standard containing a normal amount of sulfate. At a level of 1.2 mg F^-/L this increase amounted to 0.05 mg/L. Since the $SO_4^=/Cl^-$ ratio in sea water is nearly constant, correction for varying sulfate concentration may be most conveniently made by preparing calibration curves at different chlorinities. In Table I and Fig. 6 are

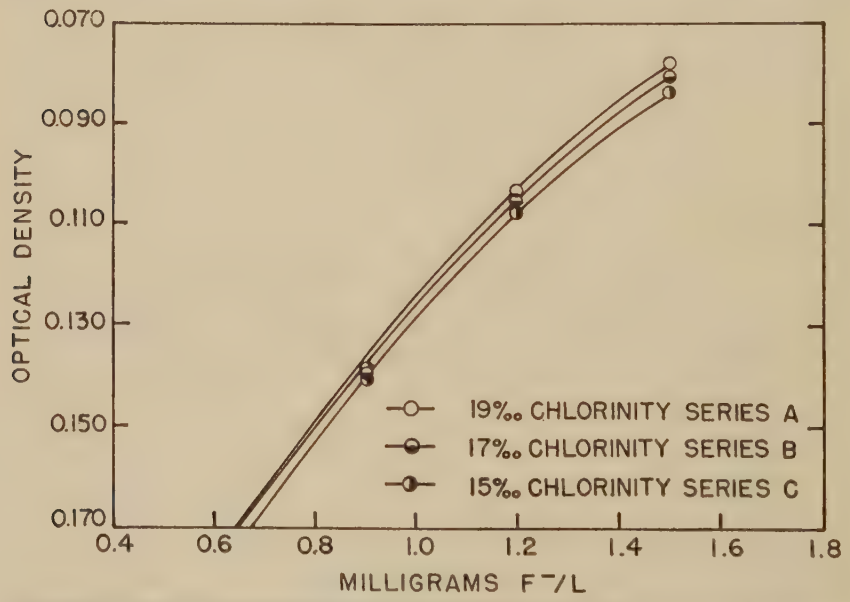

Figure 6. Effect of chlorinity upon the color development at various fluoride concentrations. Optical density observations at 512 mμ.

shown data for chlorinities 15, 17, and 19°/₀₀. Below 0.9 mg F^-/L the sulfate effect, between chlorinities of 15 and 19°/₀₀, is less than the experimental error. At a fluoride level of 1.5 mg F^-/L, the effect between chlorinities of 15 and 19°/₀₀ amounts to 0.05 mg F^-/L. The

sulfate effect as determined here for Lamar's reagent is only about one-fifth that observed for Thompson and Taylor's reagent. In many cases a single calibration curve for Cl = 17°/₀₀ would be sufficient, but for maximum accuracy it would be desirable to know the chlorinity of the water and to use the appropriate calibration curve.

TABLE I. Calibration Data for Varying Concentrations of Fluoride at Different Chlorinities

Fluoride Content (*mg/L*)	*Optical Densities*		
	Cl = 15 °/₀₀	*Cl = 17 °/₀₀*	*Cl = 19 °/₀₀*
0	0.2475	0.247	0.2465
	.248	.2475	.247
	.250	.2485	.247
0.9	0.139	0.138	0.137
	.139	.140	.139
	.1415	.140	.1385
1.2	0.1075	0.107	0.104
	.1085	.107	.108
	.107	.107	.103
1.5	0.083	0.081	0.078
	.083	.080	.078
	.082	.083	.079

Average deviation of a single determination, Cl = 17 °/₀₀	0.6 μg
Standard deviation of a single determination, Cl = 17 °/₀₀	0.9 μg
Probable deviation of a single determination, Cl = 17 °/₀₀	0.6 μg

Effect of Borate Interference. Borate is known to be present in sea water in considerable quantities. It was thought possible that borate might interfere with the fluoride determination through the formation of the fluoborate ion, BF_4^-. Preliminary experiments with large amounts of borate indicated appreciable interference. With a borate concentration of 100 mg boric acid per liter, which is four times the amount occurring in sea water, a fluoride concentration low by about 0.03 mg F^-/L was observed. This would mean that with a borate concentration equal to that in sea water the error would be within the experimental error.

Effect of Ferric Ion Interference. Similarly it was thought desirable to test for possible interference of the ferric ion through the formation of the fluoferrate ion, $FeF_6^{\equiv}$. An iron concentration of 0.1 mg Fe^{+++}/L, which is five times that present in sea water, was used to test its effect.

No interference was noted when the fluoride content was 1.5 mg/L, but high fluoride values were noted for the low concentrations, and in the case of the blank it amounted to 0.06 mg F^-/L. This, of course, is just the opposite effect to that caused by the removal of fluoride through the formation of the fluoferrate. Since the iron concentration in the sea is only one-fifth of that used in this experiment, the interference would be within the limits of measurement, even if all the iron were available as ferric ion, which seems to be contrary to fact.

Effect of Phosphate Interference. Likewise using 5.4 mg $Na_2HPO_4 \cdot 12H_2O$ per liter, which is five times the maximum phosphate present in sea water, no effect was noted at a level of 1.2 mg F^-/L, but the results were low by 0.02 mg F^-/L at 0.9 mg F^-/L and 0.07 mg F^-/L with no fluoride present. At concentrations of phosphate normally present in sea water the interference would be negligible.

Reproducibility of Results. The precision of this determination may be ascertained from the results listed in Table I. The average deviation, standard deviation, and probable deviation for a single determination have been calculated for the series Cl = 17 °/₀₀ (Table I). The probable deviation for a single determination corresponds to 0.0006 mg F^-/L.

The deviation determined in Table I is quite a bit less than the 0.02–0.05 mg F^-/L variation in the observations reported by Thompson and Taylor (6). This emphasizes the greater accuracy of measurement attained with the spectrophotometric method.

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RELATION OF FISHES AND OTHER ORGANISMS TO THE SCATTERING OF UNDERWATER SOUND

By

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ABSTRACT

An analysis of concurrent sound-scattering records and biological collections in the sea show a correlation of the deeper and more intense scattering of sound with fishes and of the upper and lighter scattering with a dominant population of euphausiids. The main group of fishes concerned are the Myctophidae, which possess all of the essential qualifications of scatterers.

INTRODUCTION

Studies of transmission of sound in the sea have revealed within the water the presence of particulate matter (scatterers) which will return a measurable echo. Bibliographies and reviews of earlier work are found in papers by Dietz (1948), Raitt (1948), Tchernia (1949), and Moore (1950). The echo sounder is one of the most fruitful means of obtaining data concerning the presence of these scatterers at various depths; with a properly adjusted instrument and a quiet sea, the record frequently shows one or more distinct traces between the bottom and surface. These intermediate traces represent the echoes returned from aggregates of scatterers. A single layer is recorded most frequently, and this has resulted in the expression "deep scattering layer." But this term is misleading, since it implies the existence of only a single layer and thereby tends to gloss over the complexity of the phenomenon. The scattering usually undergoes a diurnal (diel) migration; in the San Diego region it is generally located between 100 and 250 fathoms during the daytime, ascends to near the surface at sunset, then returns to the depths again at sunrise. Fig. 1 shows a representative record of a 24-hour cycle of the scattering behavior as recorded by a group from the U. S. Navy Electronics Laboratory under the direction of E. C. LaFond. Attention is directed especially to the correlation between the evening ascent and sunset and to the correlation between the morning descent and sunrise. The sea bottom was recorded on the second cycle, and therefore the depth is read as 400 fathoms plus the indicated value. Recent studies have shown that

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such scattering is of widespread occurrence in the Atlantic Ocean (Hersey and Moore, 1948), in the Pacific Ocean (Dietz, 1948), and in the Indian Ocean and in the Mediterranean and Red seas (Tchernia, 1949).

It is generally assumed that the scattering is of biological origin, since its behavior parallels that known for many marine organisms and since such behavior is exhibited by none of the physical features of the water. Correspondence between the diurnal migration of the scattering and of zooplankton has been established by comparison of echo-sounding records with the known behavior of many zooplankters; also, it has been established as a result of simultaneous sound records and collections. Johnson (1948) first noted the relationship between the scattering and general planktonic stratification. Hersey and Moore (1948) stressed the correlation between the behavior of the scattering and that of euphausiids, and hence these authors tentatively proposed euphausiids as the causative agents, a relationship developed further by Moore (1950). Boden (1950) analyzed about one hundred plankton samples taken between San Diego and the Pribilof Islands, the majority of which had been collected by the present author for the U. S. Navy Electronics Laboratory; concurrent sound data were available for many of the samples, and Boden concluded that euphausiids are an important constituent of the scattering, possibly the cause of it.

Nekton have also been suggested as being responsible for the layer. Chapman (1947) noted that the abraded tails of louvar are indicative of dense schooling and, therefore, of large populations. Lyman (1948) suggested squid on the basis of their vertical migration; he assumed dense populations and widespread distribution because of the appetites and distribution of their predators, among which he includes certain of the whales, fur seals, and sea birds. Both of these proposals are based on indirect evidence; such data as are available from net hauls are negative, but on the other hand both forms would be difficult to catch.

The identification of these echo-producing organisms is dependent upon definite and absolute correlation of simultaneous records of the scattering with collections of the total populations therein. This absolute correlation has not been achieved with satisfactory frequency as yet because of a complex of operational uncertainties. The echo-sounding equipment must be specifically adjusted to record the phenomenon; from the author's own experience, this is primarily a matter of regulation of sensitivity, bias, and ping length.

Collection of representative samples of organisms from the layer is complicated by two major factors: (1) All collecting nets are of neces-

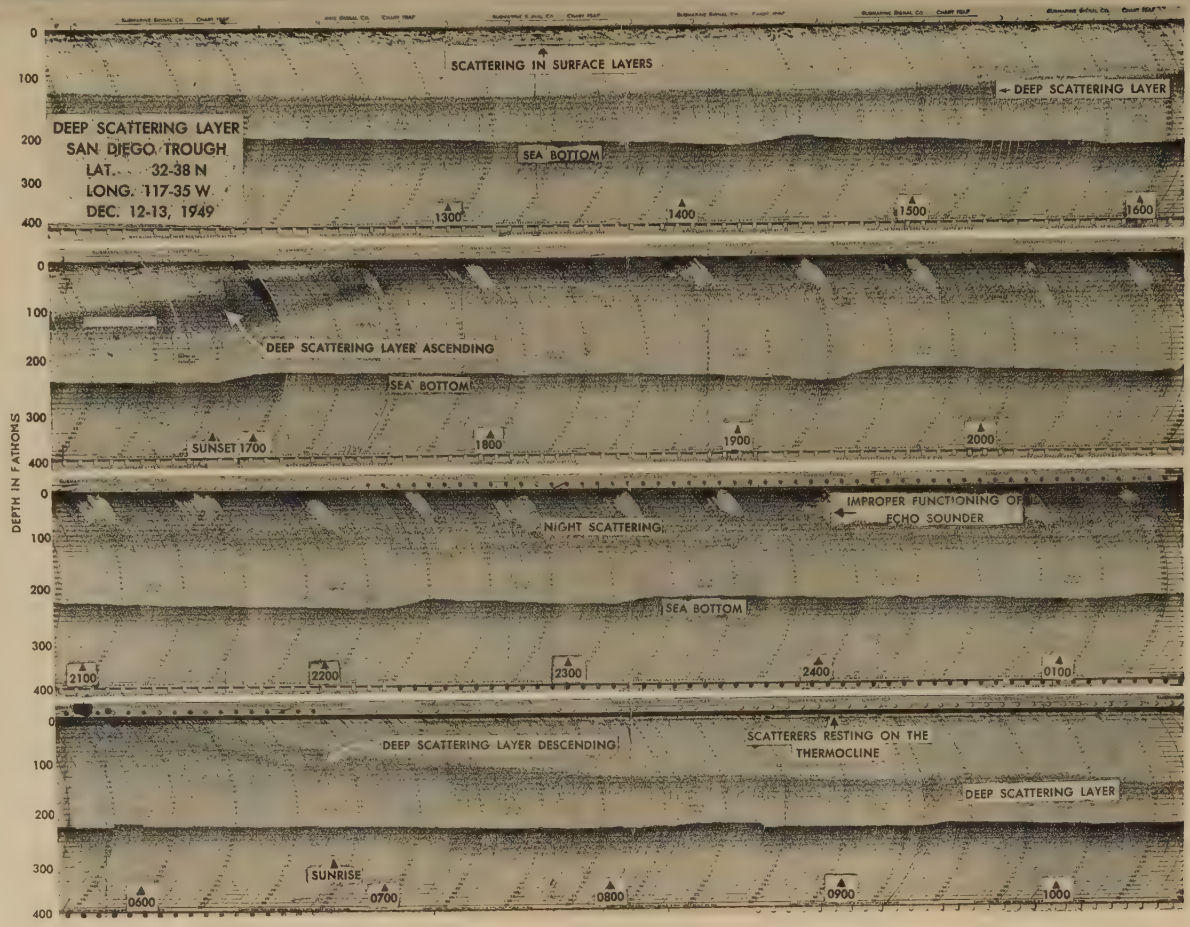

Figure 1. Fathogram for a 25-hour period illustrating diurnal migration of scattering.

sity selective rather than all inclusive; (2) the operational depth is not known with certainty until the gear has been brought aboard, and then only if a depth recorder has been employed. These aspects of the problem will be considered further in subsequent sections.

The Electronics Laboratory has made extensive collections with plankton and fish nets of various grades and sizes; hauls have been made both vertically and horizontally and, whenever possible, they have been correlated with fathogram data and with accurate depth determinations.

The plankton catches confirm the general picture of planktonic stratification during day and night; conspicuous among the planktonic forms are the euphausiids; a detailed analysis of the euphausiid data has been made by Boden (1950).

The present report deals primarily with the fish caught in a series of hauls in the Bering Sea Basin during the summer of 1949 and with collections from two of the six cruises made in the San Diego Trough during the summer of 1950; an analysis of material taken during the other four cruises is in progress. Evidence will be presented which will indicate that fish must be regarded as prominent possibilities among the potential scatterers, especially with respect to the deeper and more intense scattering. This is of particular interest in that there is some question as to whether zooplankters are of adequate size and of sufficient difference in density with sea water to act as effective reflectors of sound; the fish to be considered are not subject to this objection. However, Moore (1950) has stated that unpublished results of preliminary experimental work at Woods Hole indicate that the euphausiids would be effective as scatterers, and Dietz (1948) reported that clouds of fine sand will also cause scattering; thus, objections to the small size of zooplankters may not be valid. The writer will note several instances in which the upper part of the scattering is correlated with a dominant population of euphausiids.

APPARATUS AND METHODS

The type of net used in the series of hauls to be considered here was designed especially for investigation of the macroplankton and nekton which could easily escape the smaller and slow-filtering plankton nets. The basic net (Fig. 2) has a mouth opening of 6 feet by 6 feet. The top of the mouth frame consists of a 6 foot bar of iron 1¼ inches in diameter, with eyes on the top and bottom of each end; the bottom bar is similar; each side consists of a 6 foot manila line which extends from one bar to the other and which is spliced into the inner eyes of each bar. The net, secured to this frame by an 8 inch strip of selvage, is 30 feet long, with the forward 15 feet of ¾ inch mesh, the hinder 15

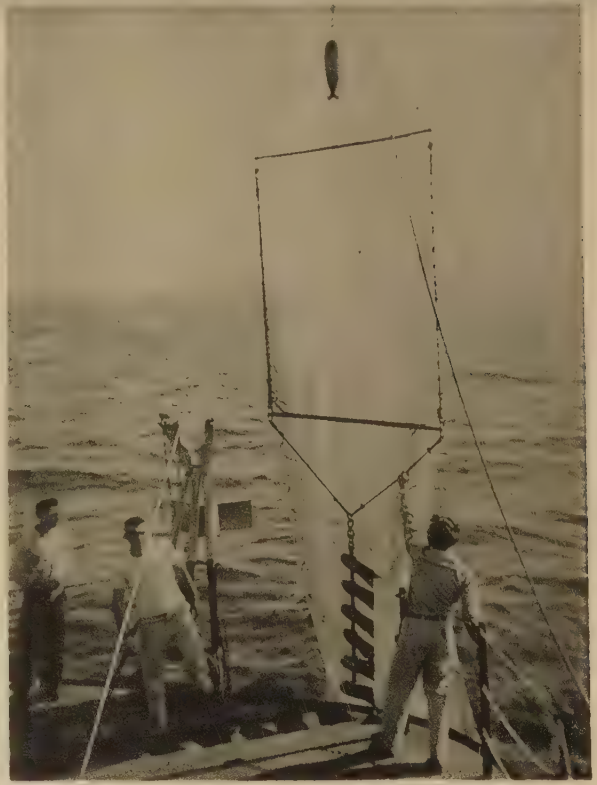

Figure 2. Net, with depth recorder attached to upper bridle and weights suspended from lower bridle.

Figure 3. Depth recorder with housing lid removed.

feet of ½ inch mesh stretched; lines of ⅜ inch diameter run from each corner of the mouth to the cod end. The original model had a canvas sleeve at the cod end, with a removable brass bucket 8 inches in diameter and 16 inches long inserted in the sleeve. Both sleeve and bucket were discarded; a liner of coarse plankton or muslin netting was added to the last five feet, and the cod end was simply tied. A short rope bridle secured the upper bar to the towing line, and weights were attached to a short bridle on the lower bar of the frame; the weights served to carry the net down and to keep the mouth open. This arrangement offers two distinct advantages: The frame is collapsed when not in use, making for convenience in handling and storage, and the mouth of the net, when fishing, is without obstruction in front of it.

A depth recorder (Fig. 3), secured to the junction of bridle and line, was used when available. The essential element is a Bourdon tube which, with changes in pressure, operates a needle that records on a chart attached to a revolving drum run by clockwork. Leakage difficulties, ultimately overcome by the use of O-rings, and collapsing due to pressure prevented us from acquiring depth records for all hauls. The type of record obtained is shown in Fig. 4. The depths obtained with the recorders were plotted against the wire lengths paid out, and

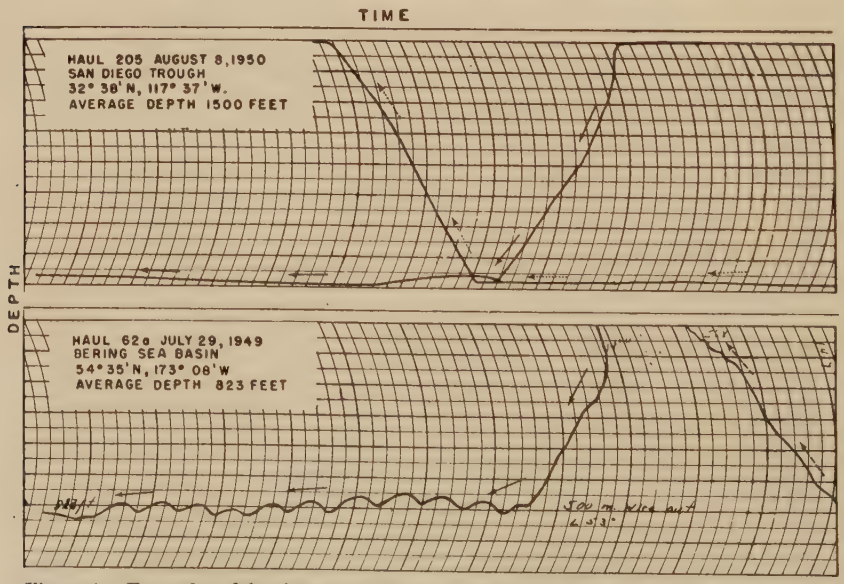

Figure 4. Examples of depth records. Depth unit interval differed for various recorders; time unit interval one minute. Arrows indicate progression of haul. Fluctuations in depth in lower record resulted from kicking engines off and on, the only way normal towing speed could be maintained in the vessel making this haul.

the resultant curve (Fig. 5) was used in determining the amount of wire to be paid out for a given depth when hauling without a depth recorder. Resultant discrepancies which would occur in horizontal hauls were minimized by the practice of making oblique hauls; that is, by bringing in the wire at a rate of five meters per minute for usually a wire length of 200 m, which was taken as the equivalent (Fig. 5) of a layer of water about 300 feet thick. Thus a layer of about this thickness would be fished in a 40 minute period. To sample scattering at 900 feet, 750 m of wire would be paid out and then brought back to 550 m of wire at a rate of five m per minute, after which it was

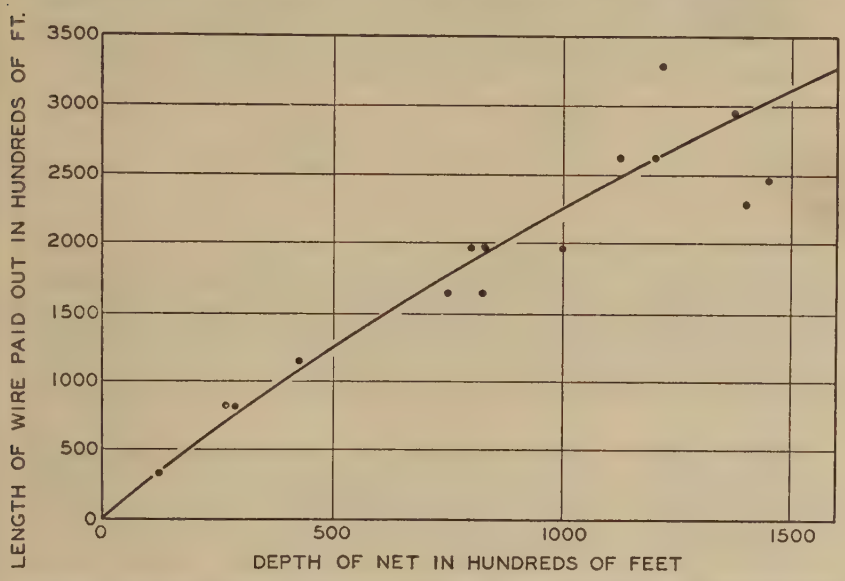

Figure 5. Relation between length of wire paid out and depth of net under standard towing conditions, determined from depth recorder data. Curve used to estimate length of wire to be paid out for a given depth when a recorder was not available.

hauled in at a rate of from 80–100 m per minute. Since the zone of scattering usually appears to be at least 120 feet thick, it is quite certain that this method insures a sampling of the zone, although the net may not be in it all of the time. Tows were made at velocities of two to three knots.

The echo-sounder equipment was the Fathometer, Model NMC-2, operated at 18 kc.

BERING SEA DATA

Table I presents the basic data from three series of net hauls in the Bering Sea Basin. For ease of comparison, the hauls in each series

are arranged according to depth. All hauls are horizontal and the depths are actual, having been obtained with the use of a depth recorder.

TABLE I.—Fish Catch in Three Series of Hauls in the Bering Sea Basin

Haul	*Time*	*Depth in feet*	*Number of fish*
	Series 1, July 29, 1949, 54° 35′ N, 173° 08′ W		
62b	1553–1623	289	0
62c	1703–1745	423	5
62a	1434–1528	823	11
	Series 2, July 30, 1949, 54° 40′ N, 172° 52′ W		
63	2013–2045	120	0
64	2332–0005	120	126
	Series 3, August 2, 1949, 55° 12′ N, 172° 38′ W		
67	0800–0845	267	2
65	0448–0532	556	9
66	0602–0650	1217	142

The hauls, except No. 64, are daytime hauls; it is assumed that conditions were fairly uniform over the period and area involved, and therefore the hauls are combined and arranged in Table II by series according to depth in order to summarize the vertical daytime distribution of the fish taken. Table II indicates clearly that, during the daytime

TABLE II.—Daytime Vertical Distribution of Fishes; from Table I

Haul	*Depth in feet*	*Number of fish*
63	120	0
67	267	2
62b	289	0
62c	423	5
65	556	9
62a	823	11
66	1217	142

and before ascent of the scattering, these fish are rare in the upper 300 feet, few in number in the next 500 feet or so, and abundant at about 1200 feet. It appears probable that there is a gradual but slight increase in numbers with depth until a level of marked concentration is reached. Fathograms at this time did not show scattering; this may have been due to one or more of the following factors: faulty adjustment of the fathometer, the disturbed state of the sea, or, as will be shown later, the fact that the echo sounder is not always an effective instrument for recording scattering at depths below 200 fathoms. Quantitative reverberation studies have frequently shown

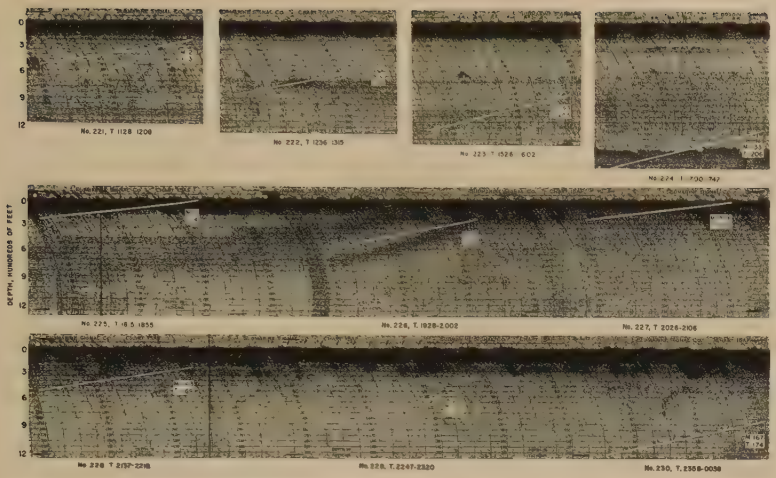

Figure 6. Relation between scattering and net hauls. Echo-sounding record read as in Fig. 1. White lines on tape represent oblique hauls. M – number of myctophids caught; T – total number of fish caught. Haul number and time are below respective hauls. Where sea bottom appears, e.g. No. 224, true depth is read at indicated value plus 400 fathoms. San Diego Trough, 32°40'N, 117°35'W, August 22, 1950.

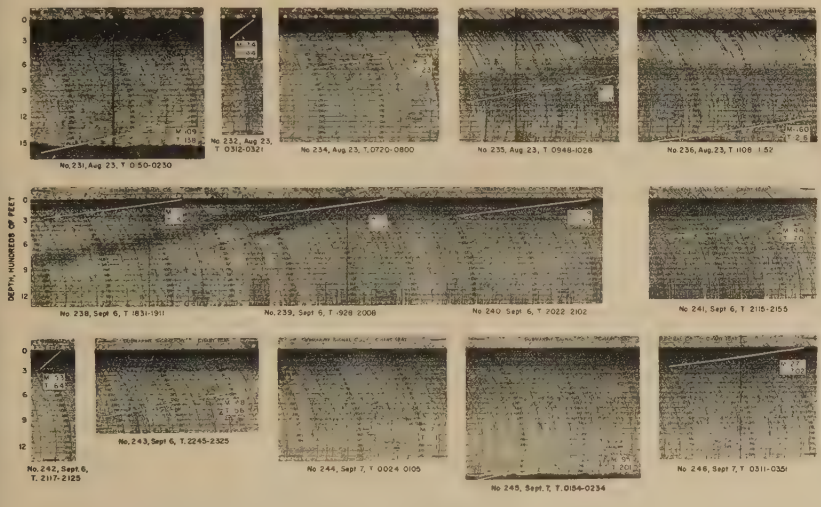

Figure 7. Relation between scattering and net hauls. Echo-sounding record read as in Fig. 1. White lines on tape represent oblique net hauls. M – number of myctophids caught; T – total number of fish caught. Haul number, date, and time are below respective hauls. Where sea bottom appears, e.g. No. 231, true depth is read at indicated value plus 400 fathoms. San Diego Trough, 32°40'N, 117°35'W.

deep scattering between 190 and 260 fathoms which were not recorded by the echo sounder; certain of these studies will be considered later in the text.

Comparison of hauls 63 and 64 (Table I) is especially interesting. Both hauls were made at 120 feet. In haul 63, made at sunset when the scattering usually appears to have completed about half of its upward migration, no fish were caught. In haul 64, made about midnight, by which time the scattering has completed its ascent, 124 fish were taken. Therefore, one may assume a potential relation between the ascent of the scattering and the population indicated by haul 64.

Table III presents a population analysis of hauls 66 and 64. Three species predominate, and the degree of agreement in numbers and composition between the catch of the deep daytime haul (66) and that of the shallow nighttime haul (64) relates the two populations. This

TABLE III.—Analysis of Fish Populations Taken in Hauls 66 (0602–0650, 1217 feet) and 64 (2332–0005, 120 feet)

Species	*Number of specimens*	
	Haul 66	*Haul 64*
Lampanyctus leucopsarus	103	55
Leuroglossus stilbius	29	42
Theragra chalcogramma	10	28
Hippoglossus (postlarva)	0	1
Totals:	142	126

correspondence, in view of the absence of such a population in the upper waters during the daytime (hauls 62b, 62c, 63, 67), indicates that we are dealing with the same population and that this population has migrated from deep to shallow water. The similarity in composition argues also against the possibility that the catches may be merely schools which were caught by chance. One may assume then that these fishes inhabit the deeper waters during the daytime and ascend to near the surface at night and that the time of their ascent is closely related to the time of the ascent of the scattering.

SAN DIEGO TROUGH DATA

Collections made in the San Diego Trough in the summer of 1950 present a picture similar to that in the southern Bering Sea. The basic data for two of the cruises (August 22–23, hauls 221–236; September 6–7, hauls 238–246) are given in Figs. 6 and 7 in which the hauls are located on the concurrent fathograms to show their relation to scattering. The net hauls of the August 22–23 cruise will be considered in some detail since a 24-hour series is available; the September 6–7 data are limited to evening and night and will be used as supplemental material.

General Scattering Correlations. The day and night vertical distribution of fish and of euphausiids for August 22–23 has been summarized respectively in Figs. 8 and 9; in these figures the relation of each block to the hauls shown in Figs. 6 and 7 is given by its corresponding number, except for the deepest hauls, which extended below

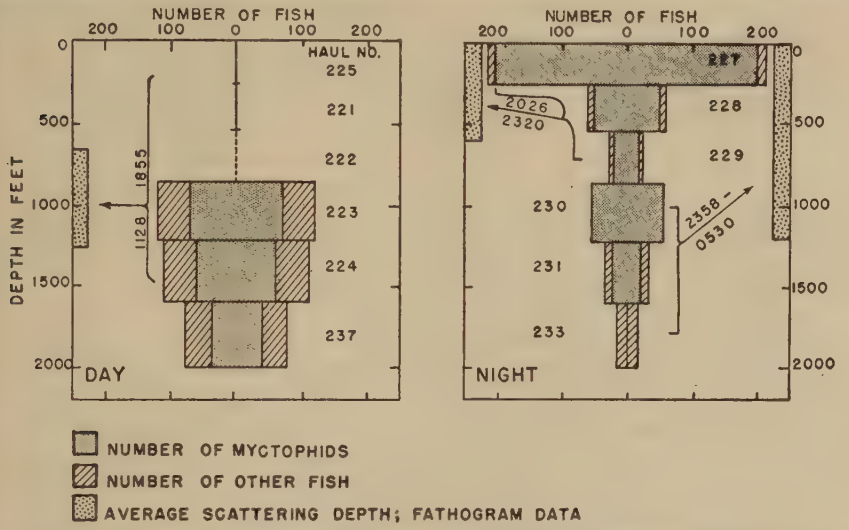

Figure 8. Day and night vertical distribution of fish and average scattering based on data shown in Figs. 6 and 7. The arrowed brackets bear the time interval during which the indicated scattering prevailed and bracket the hauls made during that period. Adjacent to each block is the haul number. San Diego Trough, August 22–23, 1950.

the bottom trace. The differences in day and night distribution are striking, and the correlation with the average scattering is good, except for the daytime euphausiid population. The average scattering is based entirely on the fathogram record, sections of which are shown in Figs. 6 and 7; daytime scattering to below 1500 feet was shown by the oscilloscope and by the visual recorder of the echo sounder. Thus scattering correlation with the daytime vertical distribution of the fish is actually better than shown. This and other relationships will be considered in more detail later in the text. In constructing the histogram for fishes we have included an assumption that the net, while traversing an upper layer before and after fishing a lower layer, would catch at least 10% of the population established in the upper layer by the standard oblique hauls at 5 m per minute. Hauls 232 and 242 (Fig. 7) were made to attempt a determination of this relationship by comparison with standard hauls at the same level; in these two instances the net was merely lowered as usual and then

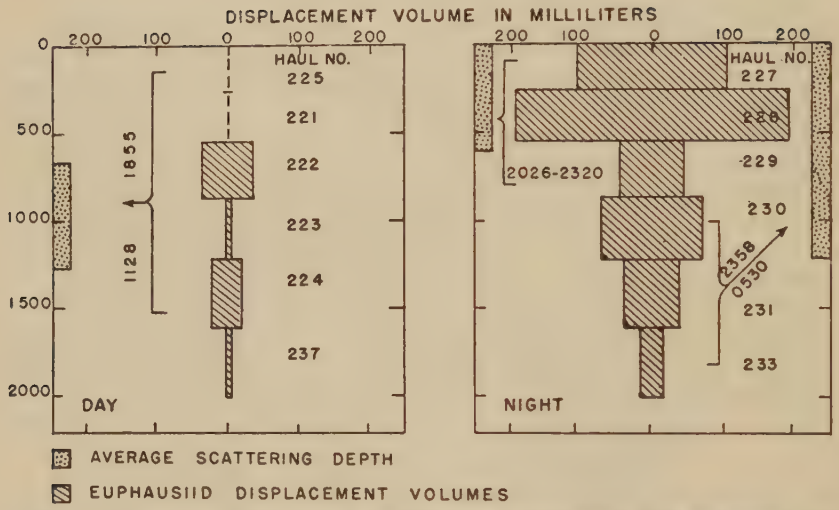

Figure 9. Day and night vertical distribution of euphausiids and scattering. The arrowed brackets bear the time interval during which the indicated scattering prevailed and bracket the hauls made during that period. Adjacent to each block is the haul number. San Diego Trough, August 22–23, 1950.

brought in immediately at the customary rate of 80–100 m per minute. Comparison of hauls 227 (Fig. 6) and 232 shows that haul 232 caught about 10% as many fish as did haul 227; haul 242 caught 38% as many as haul 240 and 21% as many as haul 246. In the first and last instances the compared hauls were about five hours apart, whereas in the second comparison the two hauls, 240 and 242, were only an hour apart; thus, perhaps more weight could be attached to the results of this latter comparison. The change in population numbers that may take place in these upper waters over a period of several hours is illustrated by comparison of hauls 240 and 246; the fish population nearly doubled and the euphausiid population almost quadrupled. In any event, the assumption of a 10% correction factor appears to be conservative. The correction has not been applied to the euphausiid graph where the comparison values were 16, 67, and 17% respectively. Obviously the matter requires further study either with repeated hauls of both types made successively or by elimination of the problem by use of closing nets.

Correlation of Ascent of Scattering with Organisms. The most striking parallelism between the behavior of the scattering and of the fish is shown by a comparison of shallow hauls immediately preceding the evening ascent with those which immediately follow it; compare hauls 225 and 227 (Fig. 6) and hauls 238 and 240 (Fig. 7). These

comparisons show that few fishes are present in the upper waters immediately before the evening ascent of the scattering, but immediately following it they are abundant; therefore, the marked increase in the number of fishes is correlated with the ascent of the scattering. However, we find the same type of increase when we consider the euphausiids. In both comparisons, few euphausiids were taken in the hauls preceding the ascent of the scattering, whereas those taken in haul 227 displaced 210 ml and those in haul 340 displaced 125 ml. Consequently, it is necessary to examine specific hauls in some detail to determine the respective relations of these two groups to the scattering.

Stratification of Scatterers. Haul 222 (Fig. 6) was taken through the upper of two layers; the lower layer is poorly defined in most of this section of the record, but it shows clearly in the preceding and following portions. Although the fish catch was negligible, 75 ml of euphausiids were taken, these constituting the bulk of the plankton; both fishes and euphausiids were scarce in the waters above, sampled in hauls 221 and 225. Consequently we relate this scattering to euphausiids. The lower of the two layers was sampled by haul 223; while the catch of euphausiids was negligible, 243 fish were taken; the lower layer of the scattering is therefore related to the fish population. Thus, consideration of hauls 222 and 223 indicates that the upper layer of this scattering is correlated with a euphausiid population, whereas the lower layer is related to the fish population.

Reverberation studies, valuable for analyzing stratification, were being conducted from an accompanying vessel, more or less concurrently with the net hauls, by the Scattering Group of the Marine Studies Branch of the Navy Electronics Laboratory. E. C. Westerfield and W. E. Batzler of this Group have prepared Figs. 10, 11 and 12, which show the relative intensities of scattering obtained from the oscillograms for the indicated hauls. The relative scattering is expressed in terms of the reverberation level (RL) corrected for the loss of intensity with depth according to the inverse square law (20 log r) and for anomalous attenuation ($2\alpha r$); scattering above 500 feet could not be read since it was "off scope," but over most of this shallow region it is at least as great as that at 500 feet. An increase of three decibels represents a doubling of the scattering intensity; in Fig. 10, for example, at 975 feet it is four times as great as it is at 775 feet or at 1200 feet.

The earliest reverberation studies on August 22 were at 1647 hours; the results at this time and at 1738 hours are shown in Fig. 10. It will be assumed that the 1647 data may be applied to haul 222 (Fig. 6) since (1) the scattering on the fathogram record at these times does not

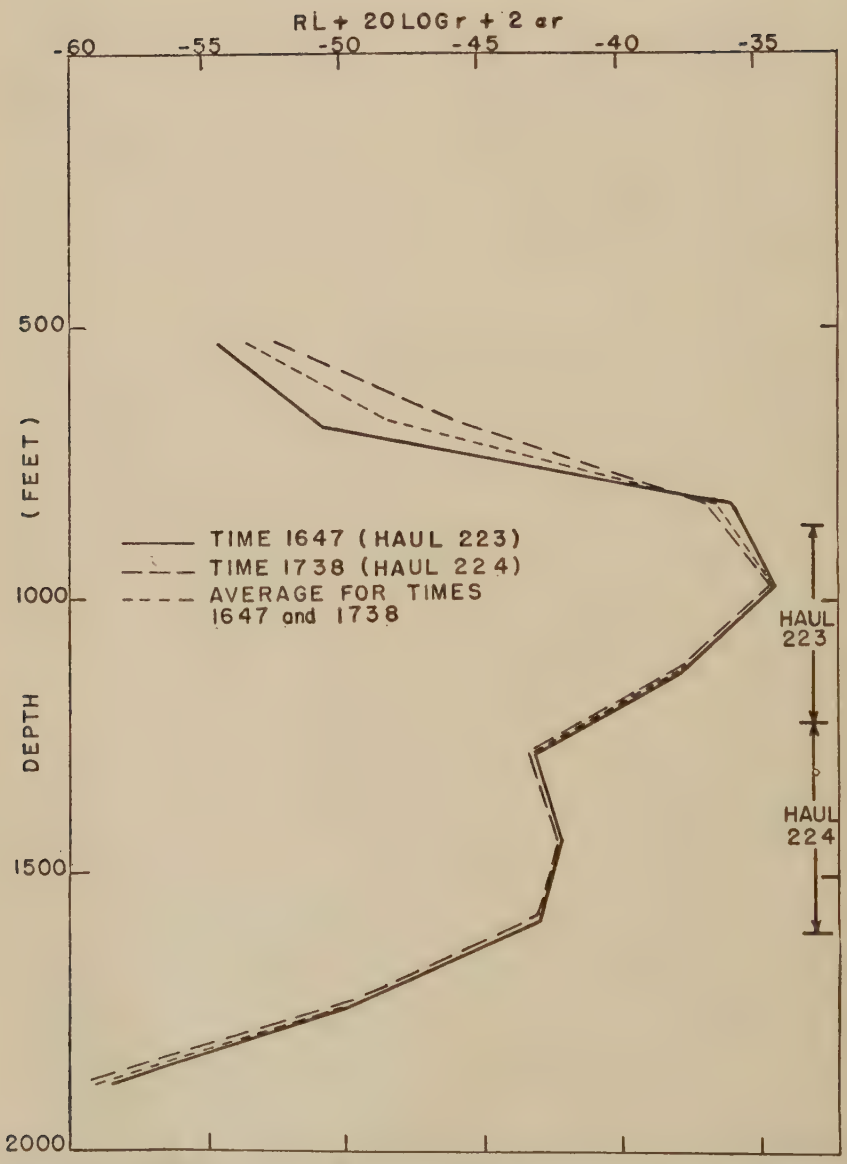

Figure 10. Relative intensities of scattering, from oscillographic recordings, and the depth relation to it of the net hauls most closely associated with the time of the recording. San Diego Trough, August 22, 1950.

differ in essentials and (2) there is negligible difference between the times 1647 and 1738, which probably indicates that we are dealing with a fairly stable condition.

If we assume, then, that the oscilloscope data for 1647 can be applied (perhaps with only minor discrepancy) to hauls 222 and 223, and if we read the relative scattering for the midpoints of these hauls from Fig. 10, we find that the average scattering sampled by haul 223 relative to the fish population is 16 times as intense as the average scattering of haul 222 (860–540 feet) relative to the euphausiid population.

It appears from the fathogram that haul 224 (Fig. 6) fished below the level of the scattering. At time 1738 (Fig. 10), however, the average scattering in the waters fished by this haul is shown to be almost four times as intense as the scattering recorded on the fathogram for the upper part (above 800 feet). But it is less than one-fourth as intense as the scattering in the waters sampled by haul 223 where the scattering was maximum, and it will be noted that the fish catch (Fig. 6) of haul 224 is less than that of 223; the difference in catch is even more pronounced, since we can assume that at least 10%, possibly as much as 35%, of the catch of haul 223 was obtained while the net was passing through the waters sampled by this haul.

Thus the daytime vertical distribution of scattering and of the related populations on August 22 may be summarized as follows: Between about 500 and 800 feet the scattering is relatively weak and associated with euphausiids; from about 800–1600 feet the scattering is markedly stronger and related to fishes, the majority of which are myctophids; the fish population has its maximum density at the level of heaviest scattering, from about 800–1150 feet (cf. Figs. 8 and 10).

Other hauls that provide information on the stratification of scatterers are 235 and 236 (Fig. 7), made the following morning. Haul 235 passed through the middle of the recorded scattering; the fish catch was negligible, but 138 ml of euphausiids were collected and therefore this scattering is related to them. In the haul below, No. 236, the fishes were abundant while the euphausiid displacement was only 28 ml; therefore scattering at this level is related to the fishes. For the most part, haul 236 was below the line of the scattering recorded on the fathogram (Fig. 7); however, the oscilloscope data for this period (Fig. 11) showed that the most intense scattering was likewise below the level of that recorded on the fathogram and that it occurred in the waters fished by haul 236; it is about four times as intense as the scattering at the level of haul 235. Thus we find again that the upper and less intense scattering, which in this instance is adequate to provide a conspicuous trace on the fathogram, is related

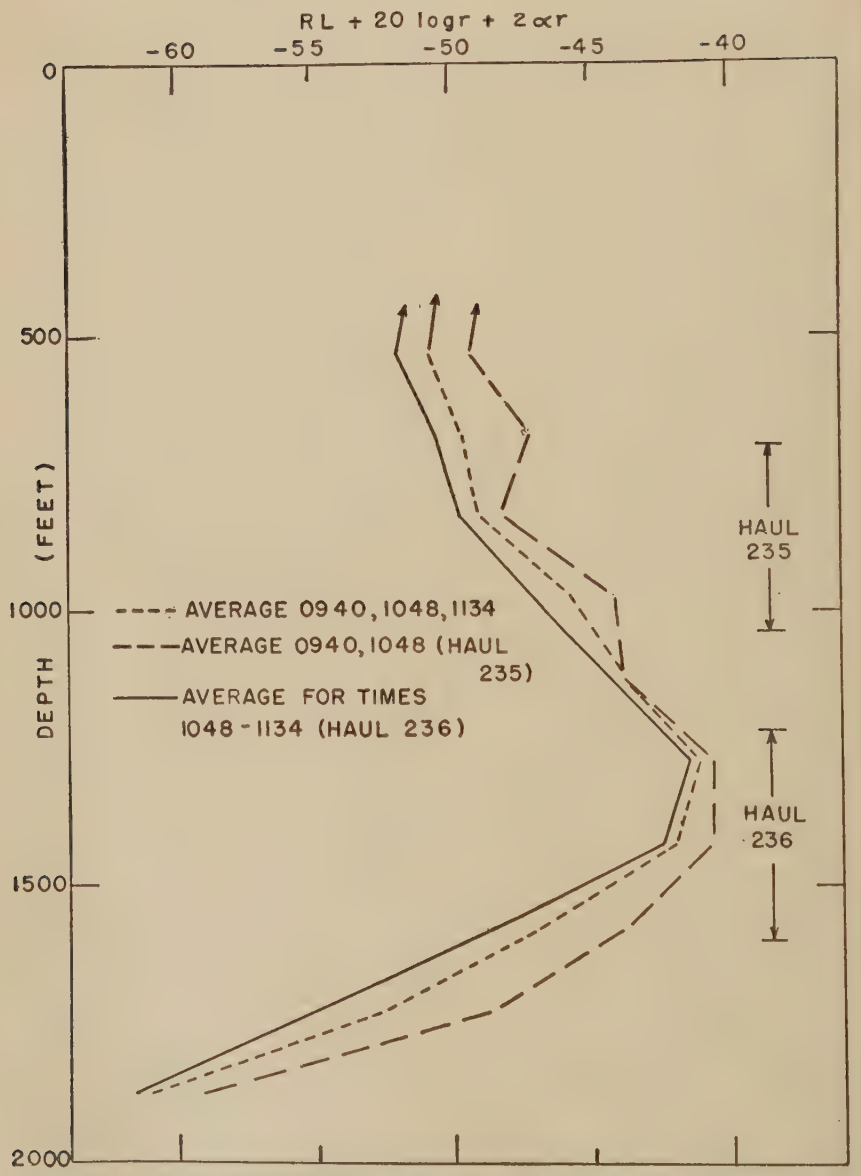

Figure 11. Relative intensities of scattering, from oscillographic recordings, and the depth relation to it of the net hauls most closely associated with the time of the recording. San Diego Trough, August 23, 1950.

to a euphausiid population, whereas the deeper and stronger scattering is associated with a fish population.

Hauls made at the time of the evening ascent (No. 226, Fig. 6; No. 239, Fig. 7) are difficult to interpret. In each instance there were double layers of scattering. It was intended that haul 226 fish the upper layer; however, the net towed chiefly between the two layers, spent about 10 minutes in the upper part of the second layer, and it would have passed through the material of the first layer while being hauled in, although the latter had merged with the surface scattering. The fact that the catch, consisting of 18 myctophids and four other fish as well as 73 ml of euphausiids, contrasts markedly (especially regarding the euphausiids) with the almost complete absence of these forms in the immediately preceding haul (225) and in an earlier haul at the same level (221) leads to the assumption that the upper layer is primarily one of euphausiids. A similar interpretation is afforded by haul 239 (Fig. 8). This haul was made through the upper layer and fished little if any through the lower layer. Comparison of this haul with 238 shows that the numbers of fish increased only negligibly whereas the euphausiids increased from none to 57 ml. Each of these hauls (226, 239) was followed by other hauls at the same level (227 and 240 respectively) after the lower layer had ascended, and each showed that the number of euphausiids increased twofold or threefold while the fish catch increased roughly twentyfold; therefore, we relate the second layer to the fish.

The considerations undertaken in this section, especially in the above paragraph, illustrate the desirability of accurate depth determinations. It is believed that the conclusions drawn are valid; the evidence, however, would be more conclusive if the depths were known precisely. An accurate depth recorder would permit horizontal hauls which would be more precise for the study of stratified scattering than are oblique hauls.

Deep Scattering at Night. It has been stated in the INTRODUCTION that the complexity of the behavior of the scatterers is becoming more evident. This is especially so with respect to the widespread belief, related to the limitations of the echo sounder, that the scatterers ascend *en toto* at sunset, remain at the surface until sunrise, and then descend. While this is obviously true for a conspicuous part of the scattering, it does not hold true for a considerable proportion; indeed, the variable behavior encountered in this study is emphatic evidence in establishing the biological nature of the phenomenon. Several related aspects will be considered.

The presence of deep scattering during the night is best seen in hauls 231 and 232 (Fig. 7) with scattering to about 1200 feet and in hauls 245

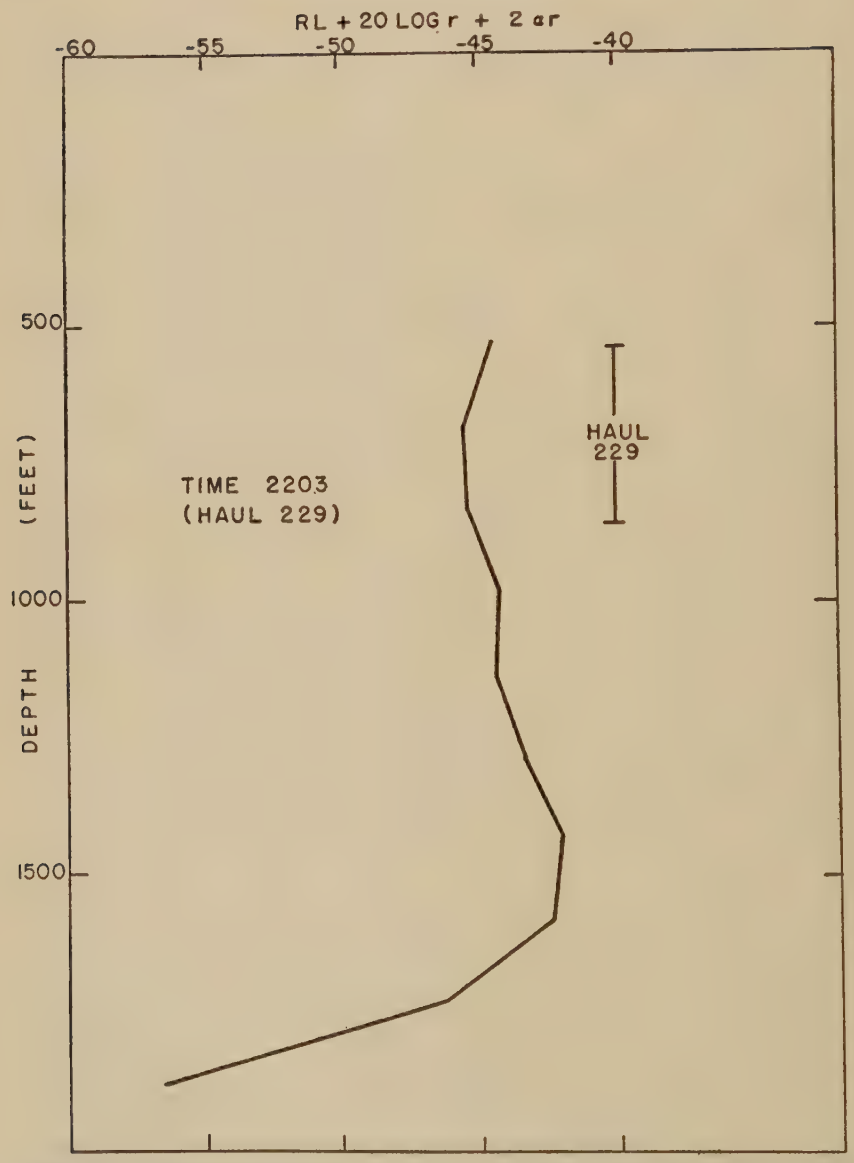

Figure 12. Relative intensities of scattering, from oscillographic recordings, and the depth relation to it of the net hauls most closely associated with the time of the recording. San Diego Trough, August 22, 1950.

and 246 with scattering to about 1000 feet; these sections represent conditions on two nights. The considerable number of fishes at these depths during the night of August 22–23 (Fig. 8) has been noted, and the same phenomenon was apparent the night of September 6–7 (Fig. 7, hauls 243–245). In hauls 229 and 230 (Fig. 7) the tape showed little scattering below 600 feet, but at 2200 o'clock[2] the record of the accompanying vessel showed dense scattering to 600 feet, moderate to sparse to 1020 feet, and slight scattering at 1200 feet; oscilloscope data at 2203 o'clock (Fig. 12) showed definite scattering to about 1600 feet, with maximum intensity (with respect to the levels below 500 feet) between 1400 and 1600 feet. A considerable euphausiid population (Fig. 9) was also present. The relative amounts of scattering due to fishes or to euphausiids at this time cannot be determined, since both forms were numerous. However, the fish distribution correlates more closely with that of the scattering intensity. The maximum scattering was in the upper 250 feet, which is where we find the greatest concentration of fishes (Fig. 8); the euphausiids, on the other hand, were concentrated at the level below. While the total scattering relative to depth shows no marked difference below 500 feet, it is perhaps significant that haul 229, which shows the lightest catch of the hauls to 1600 feet on this night (Figs. 8 and 9), corresponds to the level of lowest relative scattering intensity (Fig. 12).

Contrary to what one would anticipate, the marked increase in the myctophid population near the surface at night is not accompanied by an equivalent decrease in the population of the deeper waters; presumably fish are moving up from waters below those fished thus far in this investigation. This phenomenon is even more marked in the euphausiid populations (Fig. 9) where the numbers present in the upper 2000 feet of water would appear to be roughly eight times as numerous at night as during the day. Considerable additional data are necessary before this aspect of the problem can be clarified. Closing nets are needed to make a detailed study; some were employed in the earlier part of this study, but the ones used were unreliable and their use was discontinued.

Two other points of interest are seen on the fathogram sections, both observable at the time of the evening ascent of the scattering. At the end of haul 226 (Fig. 6), at the point where the fish count has been placed, faint scattering can be observed to swing down from the ascending scattering and then reascend at about the end of haul 227. In the region of the other evening ascent, between hauls 238 and 239 (Fig. 7), there is faint scattering at 900 feet, at which level it remains and does not share in the evening ascent. No explanation is apparent

[2] Unfortunately at this time the echo sounder was stopped for the night.

for the first case; presumably the second case concerns a group which does not take part in the evening ascent.

DISCUSSION

It remains for us to consider whether or not these forms meet the characteristics of scatterers. The basic requirements are as follows: (1) The forms must possess qualities which would be effective in scattering sound, (2) undergo diurnal migration, and (3) be abundant and widely distributed. The requirements will be considered briefly as a group for the euphausiids; for the fishes they will be considered individually and in detail.

Euphausiids. As indicated earlier, the capacity for reflection of sound by euphausiids is uncertain. However, Boden (1950) has presented some correlations between scattering and euphausiids, and the present report correlates the upper part of the scattering with these forms. Moore (1950) has considered exhaustively the matters of diurnal migration, abundance, and distribution; he finds sufficient agreement between the characteristics of the scattering and of the euphausiids to conclude that, if it can be demonstrated experimentally that the euphausiids meet the acoustical requirements, there will be little reason to consider other forms, and he refers specifically to fishes and squid.

The validity of thus eliminating other organisms from the list of potential scatterers appears questionable, even though some may appear to be unlikely. Squid, for example, were never taken in numbers sufficient to account for the scattering. Although several hundred were collected, usually fewer than six were taken in any haul and seldom were any more than two inches in body length; yet it would be surprising if the net employed would catch the larger and very active species. Again, sergestids and other types of shrimp, mysids, and amphipods were present in moderate numbers in quite a few of the hauls associated with scattering, especially at the deeper levels.

Finally, the role of fish has been emphasized in this report, and therefore the remainder of the discussion will consider these forms, with emphasis on the Myctophidae, which were the most abundant (Fig. 8). A detailed analysis of the collections is in progress and results will be reported at a later date. Thus far 53 species, representing 45 genera and 27 families, have been identified; 36 of the species are described as deep-water forms.

Scattering Qualities. The fishes caught in the Bering Sea (Table III) ranged from about 1–8 inches in length and averaged about 4

inches; the range in the San Diego Trough material was greater, but the majority were between 2–4 inches long. This size appears adequate to produce effective scattering. In addition, while the adults of many of the species probably do not exceed several inches in length, there are some that attain considerable size. Clemens and Wilby (1946) state that *Theragra chalcogramma* (represented in the Bering Sea material) attains a length of three feet; although none over a couple of inches in length was taken, and although the presence of young forms does not assure the presence of adults at the same level, the net used is ineffective for large fish and their absence from the catch does not mean that none were present.

The effectiveness of some of these forms as scatterers is increased markedly by the presence of air bladders, which would present a sharp interface from which sound waves would be scattered effectively. The data on air bladders are limited, but bladders have been ascertained for certain of the groups. The most abundant of the forms collected are the Myctophidae, nine species of which have been identified thus far. Berg (1947) stated than an air bladder is present in the family, which consists of over 100 species. Rolf Bolin of Stanford University has informed me that N. B. Marshall,[3] Esq. of the British Museum (Natural History) has examined 24 species of the family and has found an air bladder quite normally constructed in 22 of the 24 species examined by him, always with an enormous gas gland and large retia mirabilia; perhaps one of the exceptions is *Lampanyctus leucopsarus*, one of the more abundant species taken in this investigation; Dr. Dixie Lee Ray of the University of Washington tells me that this species has an air bladder which is largely filled with a type of fatty material, the exact nature of which has not been fully determined. Twenty-seven deep-water species other than myctophids were taken; according to Berg (1947), 9 possess air bladders, 3 do not, and no data have been located for the other 15.

Diurnal Migration. This characteristic has been adequately discussed in the preceding section. The details for the various species remain to be established, but this characteristic seems to be common for all of the species of Myctophidae.

[3] Since submitting this paper for publication, the writer has read the galley of a paper by N. B. Marshall, "Bathypelagic Fishes as Sound Scatterers in the Ocean," which will have appeared in the preceding issue of this Journal. The data on air bladders are especially significant. The three major families in which he establishes prevalence of air bladders were the most abundant in the writer's collections. An excellent consideration of the possible relation of scattering to bathypelagic fishes is presented.

Abundance and Distribution. The Myctophidae are among the most widely distributed and commonest of the fishes of the high seas (Bigelow and Welsh, 1925; Jordan and Everman, 1903); of the other species, we may assume that at least the 27 deep-water species have fairly wide distribution. Quantitative data are not available, but it is believed that large populations exist and that failure to demonstrate their presence has resulted primarily from limited investigation with suitable equipment. For example, Bolin (1939), in his review of the myctophid fishes, based his account of *Lampanyctus leucopsarus* upon 156 specimens from 33 stations, these having been obtained over a period of years by various agencies. Only three of these specimens were from the Bering Sea, where the author netted 158 specimens in two hauls. In the San Diego Trough hauls which have been considered, *L. leucopsarus* was the dominant myctophid; 12 of the hauls associated with scattering averaged 132 specimens per haul, and one of them (haul 227, Fig. 6) netted 372 specimens. These considerable catches are undoubtedly the result of the use of special gear correlated with the behavior of the scattering.

An indication of the abundance of the fishes may be obtained by estimating the numbers which may be assumed to be present in the sound cone on the basis of the population which was sampled. For example, in haul 223 (Figs. 6 and 10) we have a population which is being related to the scattering at this level. This was an oblique haul from 1240–860 feet, made over a period of 36 minutes at a velocity of about 2 knots, and 243 fish were caught. The volume of water sampled was about 252,000 ft^3; there were present, then, about .001 fish per cubic foot or about 0.034 fish per m^3. The volume of water in the sound cone from 1240–860 feet is approximately 55,000,000 ft^3. Thus, there would be present in the sound cone at this level about 55,000 fish. Fig. 10 shows that the depth of the population sampled in haul 223 corresponds with the level of most intense scattering; Fig. 9 shows that the euphausiid population at this level was negligible; and therefore we relate the scattering to the large population of fishes. In view of the inadequacies of the sampling gear mentioned earlier, and of the conservative estimate of the sound cone, this can be regarded as a minimum count; in addition, a considerable population may be assumed to be present in the stratum of water below, sampled by haul 224.

CONCLUSION

The evidence presented indicates that euphausiids are correlated with the upper and less intense scattering whereas fishes are related to the deeper and more intense scattering. Foremost among the latter

are the Myctophidae; they possess characteristics suitable for the reflection of sound, and, by means of concurrent sound records and collections, they have been shown to be associated with the regions of most intense scattering; they undergo a diurnal migration paralleling that of the scattering, are widely distributed, and are present in large numbers.

The entire problem of the biological nature of the scattering material requires extensive ecological study. Most of the work reported to date has failed to meet the rigid requirements of correlation of simultaneous sound records and adequate sampling at definitely determined depths. The various hypotheses thus far presented appear to tend toward the selection of one or another of the various forms known to possess at least certain of the essential qualifications. The writer believes, however, that the scattering probably represents a rather heterogeneous population, the members of which differ in relative abundance with time, depth, and locality; the main scatterers, however, appear to be the fishes and crustacean macroplankton, the euphausiids being prominent among the latter.

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HALOGENATED AMINO ACIDS OF THE BATH SPONGE[1]

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ABSTRACT

The amino acid composition and halogen content of the bath sponge, *Spongia officinalis obliqua* (D. & M.), collected in the Gulf of Mexico, was studied to determine the nature of the halogenated amino acids present. The sponge was found to contain 1.06% tyrosine, 0.38% iodine, and 0.80% bromine. It is believed that the major halogenated amino acids are the mono- (3-) and di- (3, 5-) bromo- and iodotyrosines.

INTRODUCTION

In 1910 Wheeler and Mendel (12) isolated an iodine-containing amino acid, 3, 5-diiodotyrosine, from the bath sponge. Previously this compound had been found in gorgonians by Drechsel (5), who named it iodogorgoic acid. In 1941 it was shown by Ackermann and Müller (2) that although halogen analyses of the "3, 5-diiodotyrosine" from the bath sponge always agreed with the calculated values, carbon analyses were too high by 5%. Furthermore, the x-ray diffraction pattern of the natural "diiodotyrosine" was different from that of the synthetic *d*, *l*-compound. Careful halogen analysis showed that it was a mixture of dibromotyrosine and diiodotyrosine in a ratio of 4 to 1. Although Ackermann and Müller were unable to separate the mixture from the bath sponge, they had no difficulties in separating synthetic mixtures. They believed that racemization, which accompanies hydrolysis, made the separation impossible.

Ackermann and Burchard (1) found that bath sponge spongin contained 0.79% diiodo- and dibromotyrosine. On the basis of their determinations of the iodine and bromine contents of unhydrolyzed spongin (1.41% I and 2.93% Br), 11.4% of such a mixture might be expected if all halogen were bound to tyrosine. Even if substantial losses are encountered during hydrolysis and isolation, this would not account for the discrepancy in the results. Furthermore, only 1–2% tyrosine was found in spongin which had been dehalogenated with

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hydrochloric acid instead of the 4.3% needed to bind all iodine and bromine. From this, Ackermann and Burchard concluded that there must be other halogenated amino acids in sponges.

The present author (6) found much less halogen than Ackermann in several sponges, some of which were closely related to those investigated by Ackermann. Roche and Lafon (7) found much less iodine than Ackermann and no relation between the iodine, diiodotyrosine and tyrosine contents; they did not investigate the bromine and dibromotyrosine contents. Therefore it seemed pertinent to reinvestigate the amino acid composition of the bath sponge and relate it to its halogen content. All experiments were carried out on the common bath sponge, *Spongia officinalis obliqua* (D. & M.), which was collected in the Gulf of Mexico by courtesy of F. G. Walton Smith and kindly identified by Willard Hartman.

METHODS

The sponges had been cleaned and dried for market but were not bleached. They were washed free of sand with water, then dried at about 100° C. and kept in a desiccator. The iodine and bromine analyses were carried out according to the methods described in a previous paper (6). The methods were checked with known amounts of diiodo- and dibromotyrosine.

The sponges were hydrolyzed with either 5N $Ba(OH)_2 \cdot 8H_2O$ or 20% HCl in a sealed tube at 100° for 24 hours. The acid hydrolyzates were concentrated to dryness *in vacuo;* small amounts of water were added and distilled off until the acid was removed. The residue was then taken up in a known volume of distilled water. The basic hydrolyzates were neutralized with CO_2, filtered, and concentrated *in vacuo.*

Nitrogen analyses were done by the Kjeldahl method.

Tyrosine was determined according to the method of Thomas (10), which is a color reaction with α-nitroso-β-naphthol. A Beckmann spectrophotometer was used for the color readings.

Aromatic and aliphatic amino acids were separated on charcoal columns (11). Darco G-60 charcoal was washed with ethyl acetate and water, after which it was dried at 100°. The charcoal was mixed with two parts of filter-paper pulp; the mixture was then made into a slurry with water and poured into a column. A solution of amino acids was poured onto the column and the column was washed with water until the eluate gave a negative ninhydrin test. The column was then washed with ethyl acetate saturated with water to elute the aromatic and other ring-containing amino acids. Nitrogen analyses showed a quantitative recovery of amino acids (98.6%).

The method of paper chromatography of Consden, Gordon and Martin (3) was employed for the separation of amino acids. Whatman No. 1 filter paper and the solvents phenol-water, collidine-water (Eastman Technical grade), and o- and p-cresol-water were used. The chromatograms were developed with a ninhydrin solution in water-saturated butanol. The amino acids were identified by comparing their flow rates with those of known compounds and by using the map prepared by Dent (4).

Diiodotyrosine was prepared according to the method of Savitzkii (9),[3] dibromotyrosine according to the method of Zeyneck (13). A sample of 3-iodotyrosine was obtained through the courtesy of R. M. Herriot.

RESULTS AND DISCUSSION

Each series of determinations was carried out on one sponge. Each determination was carried out several times, and each series was done on several sponges of the same species collected in the same general area. The figures given are means of these values.

In a typical experiment, 0.51 g. dried sponge was hydrolyzed with hydrochloric acid to dehalogenate tyrosine. During hydrolysis some material is generally lost, some of the loss being due to an actual decomposition of amino acids and some to mechanical losses. To ascertain the amount lost, nitrogen determinations were done on the unhydrolyzed sponge and on aliquots of the hydrolysate. It was found that the total sponge had 15.4% N, whereas the hydrolysate had the equivalent of 14.5% N. Tyrosine determinations were carried out on the hydrolysate, and the values were corrected for the nitrogen loss (5.8%). It was found that the sponge had an average of 1.06% tyrosine (including halogenated tyrosine).

Iodine and bromine analyses were done on a part of the above sponge. A mean of five analyses gave 0.38% I and 0.80% Br.

The above figures indicate that 1 gram of this bath sponge has 0.0587 millimols tyrosine, 0.10 millimols bromine, and 0.0294 millimols iodine (0.129 millimols halogen), or about two halogen molecules for each tyrosine molecule. In this sponge, therefore, almost all of the halogen molecules could be bound to tyrosine.

Amino acid analyses were done on the barium hydroxide hydrolysate of the sponge. This method racemizes the amino acids but does not dehalogenate tyrosine; the amino acids serine, arginine, cystine and cysteine, and threonine are destroyed to a large extent. Paper chromatography with phenol and collidine showed the presence of

[3] The author wishes to express her thanks to Jane Setlow, who translated part of the paper.

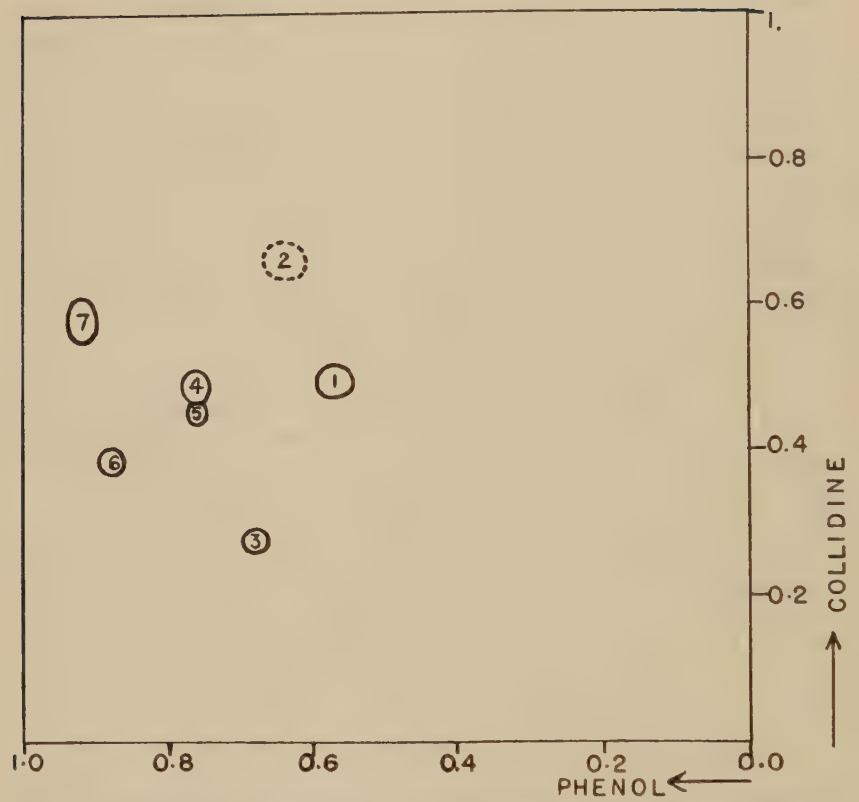

Figure 1. Schematic representation of a paper chromatogram of the "aromatic" fraction of a basic hydrolysate of the bath sponge. 1 = tyrosine; 2 = 3-iodotyrosine; 3 = histidine; 4 = dibromotyrosine; 5 = tryptophan; 6 = phenyl alanine; 7 = diiodotyrosine.

aspartic acid, glutamic acid, lysine, glycine, alanine, proline and hydroxyproline, α-aminobutyric acid, leucine, valine, methionine, and tyrosine.

The most important amino acids that could combine with iodine are the aromatic amino acids and histidine. Since they are present in much smaller amounts than the aliphatic amino acids, they cannot be seen easily on the chromatograms. Therefore the amino acids were first separated on charcoal and each fraction was chromatographed on paper.

The chromatograms of the aliphatic fraction showed no amino acids which had not been on the chromatograms of the total hydrolysate. The aromatic fraction gave seven spots when a concentrated

solution was chromatographed with phenol and collidine; they could be identified as tyrosine, monoiodotyrosine, diiodotyrosine, dibromotyrosine, phenylalanine; the very faint spots are probably tryptophan and histidine (see Fig. 1). The flow rates of diiodo- and dibromotyrosine are similar (see Table I), and they were separated only when large sheets of paper were used. The monoiodotyrosine spot was always diffuse. No obvious indication of a spot that might be referable to iodobromotyrosine was observed, though the occurrence of such a compound would surely be expected.

Since Roche, Rand and Yagi (8) recently found monobromotyrosine in some gorgonians, it is not unlikely that it is present also in sponges. No pure bromotyrosine could be obtained; it is probable, however, that it has similar flow rates to those of iodotyrosine in phenol and collidine and that the spot labeled monoiodotyrosine is really a mixture of iodo- and bromotyrosine. Roche and co-workers found that the flow rates for the two were similar in butanol containing acetic acid or ammonia.

The present author was unable to find any differences in the behavior of racemized and nonracemized diiodo- and dibromotyrosine mixtures. Mixtures of synthetic dibromo- and diiodotyrosine, when chromatographed, had the same flow rates whether they were racemized with barium hydroxide or not. They could be easily separated with o-cresol and with phenol and collidine when allowed to run for a long time. Table I gives the flow rates (R_f values) in several solvents.[4]

TABLE I. Flow Rates of Tyrosine and Some Halogenated Tyrosine Derivatives

Solvent	R_f					
(*water-saturated*)	*Tyrosine*	*3-Iodotyrosine*	*Nonracemized Mixture*		*Racemized Mixture*	
			Diiodotyrosine	*Dibromotyrosine*	*Diiodotyrosine*	*Dibromotyrosine*
Phenol	0.60	0.64	0.90	0.76	——	——
o-Cresol	0.17	——	0.72	0.53	0.71	0.57
p-Cresol	0.33	——	0.82	0.74	——	——
Collidine	0.50	0.66	0.62	0.49	——	——

From the experiments presented in this paper it appears likely that diiodotyrosine, dibromotyrosine, monoiodotyrosine, and probably monobromotyrosine are the main halogenated amino acids in the bath sponge, *Spongia officinalis obliqua*, from the Gulf of Mexico. The tyro-

[4] Absolute figures are of little value unless all observations are made at the same time [see Dent (4)]. In these experiments, all amino acids were run in the same solvent simultaneously.

sine and halogen determinations indicate that all halogen could be attached to tyrosine. But the possibility exists that there are small amounts of other halogenated amino acids. The most likely amino acids to take up halogen would be histidine and phenyl alanine. However, preliminary experiments with radioactive iodine did not give any indication of their presence.

The author wishes to thank Werner Bergmann for stimulating her interest in sponge chemistry, Harold G. Cassidy for his help with the amino acid chemistry, and G. Evelyn Hutchinson for his constant help, interest and encouragement.

SUMMARY

The bath sponge, *Spongia officinalis obliqua* (D. & M.), collected in the Gulf of Mexico, was found to contain 1.06% tyrosine, 0.38% iodine, 0.80% bromine, and 15.4% nitrogen.

The amino acid composition of the sponge was studied with special emphasis on the halogenated amino acids.

It is believed that the major halogenated amino acids are the mono- (3-) and di- (3, 5-) bromo- and iodotyrosines.

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